


Declarations under Rule 4.17:
— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iv))
— if inventorship (Rule 4.17(iv))

Published:
— with international search report (Art. 21(3))

Title: METHODS FOR TREATING PROTEINOPATHIES

FIG. 1

Abstract: This disclosure relates to a method of treating a proteinopathy in a subject, the method comprising administering to the subject an effective amount of a quinuclidine compound. The disclosure also relates to a method of reducing, reversing or preventing the accumulation of protein aggregates in tissue of a subject diagnosed as having a proteinopathy, or being at risk of developing a proteinopathy, the method comprising administering to the subject an effective amount of a quinuclidine compound. Also disclosed is a pharmaceutical composition comprising a quinuclidine compound for use in said methods. The proteinopathy may be a synucleinopathy or a tauopathy, such as Parkinson's disease, Alzheimer's disease or dementia with Lewy bodies.
METHODS FOR TREATING PROTEINOPATHIES

This application claims the benefit of priority to U.S. Provisional Application No. 62/131,071 filed March 10, 2015, the disclosure of which is incorporated herein in its entirety.

This disclosure relates to methods for treating proteinopathies and to quinuclidine compounds for use in said methods. The disclosure relates particularly to the oral administration of quinuclidine compounds for treating tauopathies and/or synucleinopathies, e.g. Parkinson’s disease.

SUMMARY OF THE INVENTION

In medicine, proteinopathy refers to a class of diseases in which certain proteins become structurally abnormal, and thereby disrupt the function of cells, tissues and organs of the body. Often the proteins fail to fold into their normal configuration. In this misfolded state, the proteins can become toxic in some way (again of toxic function) or they can: an lose their normal function. The proteinopathies include diseases such as Alzheimer’s disease, Parkinson’s disease, amyloidosis, and a wide range of other disorders.

Proteinopathies are widespread throughout the population. For example, nearly one million people in the US are living with Parkinson’s disease and as many as 5.1 million Americans have Alzheimer’s disease. There are currently no cures for these diseases, and many of the molecular mechanisms underlying the disease and progression of the disease are unknown.

Tauopathies form one particular class of proteinopathies. These are a collection of neurodegenerative disorders characterised pathologically by the presence of aggregates of phosphorylated tau protein, typically in the form of neurofibrillary tangles or Pick’s bodies. These disorders are age-related and are often, to a greater or lesser extent, inherited. For example, mutations in MAPT (encoding the microtubule-associated protein tau in humans, located on chromosome 17q21) account for around 30% of inherited cases of frontotemporal dementia. Several human tau isoforms are known to be generated by alternative splicing of MAPT, and mutations in this gene can result in altered levels of these isoforms, which may lead to protein aggregation and disease progression.
Another class of proteinopathies is characterised by structurally abnormal a-synuclein proteins. These diseases are known collectively as synucleinopathies. a-synuclein is a protein encoded by the SNCA gene in humans. In particular, a-synuclein can aggregate to form insoluble fibrils in pathological disorders characterized by Lewy bodies. These disorders include, for example, Parkinson's disease and Lewy body dementia.

Tau- and a-synuclein-associated pathologies are frequently found in tandem in patients with Parkinson's disease and in patients with Lewy body dementia. In these cases, the diseases may be characterized both as tauopathies and as synucleinopathies.

Although there are no cures for these devastating diseases, there are a number of small molecule drugs available to help alleviate the symptoms of some proteinopathies. However, there is a real need in the art to develop therapeutics effective in alleviating or managing the symptoms associated with proteinopathies, especially proteinopathies such as Parkinson’s disease and Lewy body dementia. There is a particular need to develop therapeutics effective in treating the underlying pathophysiology of proteinopathies.

The present inventors have determined that certain quinuclidine compounds can reduce, reverse or prevent protein aggregation in tissues of subjects with proteinopathies. The inventors have also determined that these quinuclidine compounds can ameliorate memory deficits in animal models of proteinopathies. These results indicate that treatments with quinuclidine compounds as described herein will be effective to treat the underlying pathophysiology of proteinopathies.

Accordingly, in a first aspect the present invention provides a method of treating a proteinopathy in a subject, the method comprising administering to the subject an effective amount of a compound of formula (I),

\[
\text{(I)}
\]

\begin{center}
\includegraphics[width=\textwidth]{formula.png}
\end{center}
or a pharmaceutically acceptable salt or prodrug thereof, wherein:

R is hydrogen, halogen, or a cyano, nitro, hydroxy, thio, or amino group, or a pharmaceutically acceptable salt or prodrug thereof,

-alkyl, C₁₋₆ -alkenyl, C₂₋₆ -alkynyl, C₁₋₆ -alkyloxy, C₂₋₆ -alkenyl, or C₂₋₆ -alkynoxy, or

C₂₋₆ -alkynoxy group, optionally substituted by one or more (e.g., 1, 2, or 3) groups independently selected from a halogen, and a cyano, nitro, hydroxy, thio, or amino group; or

C₂₋₆ -alkyl, C₁₋₆ -alkenyl, C₂₋₆ -alkynyl, C₁₋₆ -alkyloxy, C₂₋₆ -alkenyl, or C₂₋₆ -alkynoxy, or

R²⁻ and R³⁻ are each independently selected from a halogen, or a nitro, to, hydroxy, thio, or amino group; and a C₁₋₆ -alkyl, or C₁₋₆ -alkyloxy group, optionally substituted by one or more halogens; or

R²⁻ and R³⁻ together form a cyclopropyl, or cyclobutyl group, optionally substituted by one or more halogens; or

R¹⁻, R²⁻, and R³⁻ are each independently selected from hydrogen, or a halogen, or a nitro, to, hydroxy, thio, or amino group; and a C₁₋₆ -alkyl, or C₁₋₆ -alkyloxy group, optionally substituted by one or more fluorine atoms, or

R¹⁻, R²⁻, and R³⁻ are each independently selected from a halogen, or a methyl, or an ethyl group, optionally substituted by a halogen, or a hydroxy, thio, or amino group.

In one embodiment, R¹⁻ is hydrogen, or a fluorine, or a methyl, or an ethyl group, optionally substituted by a halogen, or a hydroxy, thio, or amino group.

In one embodiment, R²⁻ and R³⁻ are each independently selected from a methyl and an ethyl group, optionally substituted by a halogen, or a hydroxy, thio, or amino group.

In one embodiment, R¹⁻ is selected from a halogen, and a C₁₋₆ -alkyl, or C₁₋₆ -alkyloxy group, optionally substituted by one or more groups selected from a halogen, and a C₁₋₆ -alkyl, or C₁₋₆ -alkyloxy group.

In one embodiment, R¹⁻, R²⁻, and R³⁻ are both hydrogen, or

R¹⁻ is fluorine, or a 2-methoxyethoxy group, and R²⁻ and R³⁻ are both hydrogen, or

R¹⁻ is fluorenyl, or a 2-(4-chlorophenyl) benzyl group, optionally substituted by one or more halogens, or

R¹⁻ is in a position on the benzene ring para to the group A₁ in a 1,3-, 3-, or a 1,4- relationship.

In another embodiment, A is a 5-membered heteroaryl group which contains 1, 2, or 3 heteroatoms selected from N, S, or O, and the groups C(R¹⁻)⁻ and -(C₅H₄[R¹⁻]R²⁻R³⁻)⁻ are attached to the group A in a 1,3-, 3-, or a 1,4- relationship.

In another embodiment, A is a 5-membered heteroaryl group which contains 1, 2, or 3 heteroatoms selected from N, S, or O, and the groups C(R¹⁻)⁻ and -(C₅H₄[R¹⁻]R²⁻R³⁻)⁻ are attached to the group A in a 1,3-, 3-, or a 1,4- relationship.
In one embodiment, the compound is a compound of formula (I), (II) or (III), or a pharmaceutically acceptable salt or prodrug thereof.

In one embodiment, the compound is a compound of formula (IV), or a pharmaceutically acceptable salt or prodrug thereof.
In one embodiment, the compound is a compound of formula (VI), (VII) or (VIII), or a pharmaceutically acceptable salt or prodrug thereof.

In one embodiment, the compound is a compound of formula (IX) or (XI),
or a pharmaceutically acceptable salt or prodrug thereof. In one embodiment, R\(^4\) is fluorine.

In particular, the compound is selected from: quinuclidin-3-yl(2-(4'-fluoro-[1,1'-biphenyl]-3-yl)propan-2-yl)carbamate; (S)-quinuclidin-3-yl(2-(2-(4'-fluorophenyl)thiazol-4-yl)propan-2-yl)carbamate; (S)-quinuclidin-3-yl(2-(4'-(2-(2-methoxyethoxy)-[1,1'-biphenyl]-4-yl)propan-2-yl)carbamate; and the pharmaceutically acceptable salts and prodrugs thereof.

In an embodiment, the proteinopathy is a tauopathy. In one embodiment, said tauopathy is selected from: Parkinson's disease, Alzheimer's disease, Lewy Body Dementia, Pick's disease, progressive supranuclear palsy, dementia pugilistica, parkinsonism linked to chromosome 17, Lytico-Bodig disease, tangle predominant dementia, argyrophilic grain disease, ganglioglioma, gangliocytoma, meningioangiomatosis, subacute sclerosing panencephalitis, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, lipofuscinosis, corticobasal degeneration, frontotemporal dementia, lobar degeneration, and Huntington's disease.
In one embodiment, said subject does not have protein aggregates comprising α-synuclein in their CNS (e.g. in neurons of the substantia nigra, cerebral cortex, hippocampus, frontal lobes and/or temporal lobes).

In one embodiment, said tauopathy is Parkinson's disease characterised by the presence of protein tau, but not α-synuclein, within protein aggregates in the CNS of said subject (e.g. in neurons of the substantia nigra, cerebral cortex, hippocampus, frontal lobes and/or temporal lobes).

In another embodiment, the proteinopathy is a synucleinopathy. In one embodiment, said synucleinopathy is selected from Lewy Body Dementia, Parkinson's disease and multiple system atrophy.

In one embodiment, said method prevents, reduces or reverses the progression of dementia in the subject.

In one embodiment, said subject is a mammal, e.g. a human.

In one embodiment, said subject has been diagnosed as being at risk of developing said proteinopathy, and the method prevents or delays the onset and/or development of the proteinopathy in the subject.

In one embodiment, said compound, or pharmaceutically acceptable salt or prodrug thereof, is administered by systemic administration, e.g. via a non-parenteral route. In one embodiment, said compound, or pharmaceutically acceptable salt or prodrug thereof, is administered orally.

In a related aspect, the invention provides a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined herein for use in a method of treating a proteinopathy in a subject. In another related aspect, the invention provides the use of a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined herein, in the manufacture of a medicament for use in a method of treating a proteinopathy in a subject. In certain embodiments, the method of treating a proteinopathy is as defined herein.

In another aspect, the invention provides a method of reducing, reversing or preventing the accumulation of protein aggregates in tissue of a subject diagnosed as having a
proteinopathy, or diagnosed as being at risk of developing a proteinopathy, wherein said protein aggregates comprise protein tau and/or α-synuclein, the method comprising administering to said subject an effective amount of a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined herein.

In an embodiment, the protein aggregates comprise aggregates of protein tau and said α-synuclein, wherein the proteinopathy is a tauopathy. In one embodiment, said tauopathy is selected from Parkinson’s disease, Alzheimer’s disease, Lewy Body Dementia, Pick’s disease, progressive supranuclear palsy, dementia pugilistica, parkinsonism linked to chromosome 17, Lytico-Bodig disease, tangle predominant dementia, Argyrophilic grain disease, ganglioglioma, gangliocytoma, meningioangiomatosis, subacute sclerosing panencephalitis, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, se, lipofuscinosis, corticobasal degeneration, frontotemporal dementia, frontotemporal lobar degeneration and Huntington’s disease.

In one embodiment, said subject does not have protein aggregates comprising α-synuclein in said tissue. In one embodiment, said tauopathy is Parkinson’s disease, se.

In another embodiment, the protein aggregates comprise aggregates of α-synuclein and said α-synucleinopathy. In one embodiment, said α-synucleinopathy is selected from Lewy Body Dementia, Parkinson’s disease, and multiple system atrophy.

In one embodiment, said method prevents, reduces or reverses the progression of dementia in the subject.

In one embodiment, said tissue is a neuron of the substantia nigra, cerebral cortex, ex, hippocampus, frontal lobes and/or temporal lobes of said subject, etc.

In one embodiment, said subject is a mammal, e.g., a human.

In one embodiment, said compound, or pharmaceutically acceptable salt or prodrug thereof, is administered by systemic administration, e.g., via a non-parenteral route. In one embodiment, said compound, or pharmaceutically acceptable salt or prodrug thereof, is administered orally.
In a yet further aspect, the invention provides a method of preventing, reducing or reversing loss of neural function in a subject diagnosed as having, or at risk of developing, a proteinopathy, the method comprising administering to said subject an effective amount of a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined herein.

In an embodiment, the proteinopathy is a tauopathy. In one embodiment, said tauopathy is selected from Parkinson's disease, Alzheimer's disease, Lewy Body Dementia, Pick's disease, progressive supranuclear palsy, dementia pugilistica, Parkinsonism linked to chromosome 17, Lytico Bodig disease, tangle predominant dementia, Argyrophilic grain in disease, ganglioglioma, gangliocytoma, meningoangiomatosis, subacute sclerosing panencephalitis, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, se, lipofuscinosis, corticobasal degeneration, frontotemporal dementia, frontotemporal lobar degeneration and Huntington's disease.

In one embodiment, said subject does not have protein aggregates comprising α-synuclein in their CNS (e.g. in neurons of the substantia nigra, cerebral cortex, hippocampus, frontal lobes, and/or temporal lobes).

In one embodiment, said tauopathy is Parkinson's disease characterised by the presence of protein (tau), but not α-synuclein, within protein aggregates in the CNS of said subject (e.g. in neurons of the substantia nigra, cerebral cortex, hippocampus, frontal lobes and/or temporal lobes).

In another embodiment, the proteinopathy is a synucleinopathy. In one embodiment, said synucleinopathy is selected from Lewy Body Dementia, Parkinson's disease and multiple system atrophy.

In one embodiment, said method prevents, reduces or reverses the progression of dementia in the subject.

In one embodiment, said subject is a mammal, e.g., a human.

In one embodiment, said compound, or pharmaceutically acceptable salt or prodrug thereof, is administered by systemic administration, e.g., via a non-parenteral route. In one
In one embodiment, said compound, or pharmaceutically acceptable salt or prodrug thereof, is administered orally.

In one embodiment, the loss of neural function comprises loss of cognitive function, autonomic function and/or motor function.

In one embodiment, the loss of neural function comprises loss of cognitive function, autonomic function and/or motor function and/or problem solving. g.

In one embodiment, the loss of neural function comprises loss of autonomic function and/or the method prevents, reduces or reverses orthostatic hypotension, constipation, dysphagia, nausea, hypersalivation, hyperhydrosis and/or urinary and sexual dysfunction.

In one embodiment, the loss of neural function comprises loss of motor function and the method prevents, reduces or reverses Parkinsonism.

In one embodiment, the method prevents, reduces or reverses motor dysfunction (e.g., tremor), bradykinesia, rigidity, postural instability and/or impaired balance.

In a related aspect, the invention provides a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined herein for use in a method of preventing, reducing or reversing loss of neural function in a subject, as defined herein. In another related aspect, the invention provides the use of a compound, or a pharmaceutically acceptable salt, or prodrug thereof, as defined herein in the manufacture of a medicament for use in an a method of preventing, reducing or reversing loss of neural function in a subject, as defined herein.

In a still further aspect, the invention provides a method of preventing, reducing or reversing the progression of dementia in a subject, diagnosed as having, or at risk of developing, a proteinopathy, the method comprising administering to the subject an effective amount of compound, or a pharmaceutically acceptable salt, or prodrug thereof, as defined herein.
In one embodiment, the method prevents, reduces or reverses early symptoms of dementia (e.g., difficulty remembering recent conversations, names or events, and/or apathy and depression). In another embodiment, the method prevents, reduces or reverses later symptoms of dementia (e.g., impaired communication, poor judgment, disorientation, on, confusion, behavior changes and/or difficulty in speaking, swallowing (and/or walking).)

In a related aspect, the invention provides a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined herein for use in a method of preventing, reducing or reversing the progression of dementia in a subject as defined herein. In another related aspect, the invention provides the use of a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined herein in the manufacture of a medicament for use in an a method of preventing, reducing or reversing the progression of dementia in a subject as defined herein.

In a yet further aspect, the invention provides a method of preventing, reducing or reversing mild cognitive impairment in a subject diagnosed as having, or at risk of developing, a proteinopathy, the method comprising administering to the subject an effective amount of a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined herein.

In a related aspect, the invention provides a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined herein for use in a method of preventing, reducing or reversing mild cognitive impairment in a subject as defined herein. In another related aspect, the invention provides the use of a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined herein in the manufacture of a medicament for use in an a method of preventing, reducing or reversing mild cognitive impairment in a subject as defined herein.

In another aspect, the invention provides a pharmaceutical dosage form comprising a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined herein; and a pharmaceutically acceptable excipient, wherein the dosage form is formulated to provide, when administered orally, an amount of said compound, salt or prodrug sufficient to prevent, reduce or reverse the accumulation of protein aggregates in tissue of a human subject diagnosed as having, or being at risk of developing, a proteinopathy.
In an embodiment, said dosage form is formulated to provide, when administered orally, an amount of said compound, salt, or prodrug sufficient to prevent, reduce or reverse the accumulation of protein tau-containing aggregates in tissue of a human subject diagnosed as having, or being at risk of developing, Parkinson's disease.

In another embodiment, said dosage form is formulated to provide, when administered orally, an amount of said compound, salt, or prodrug sufficient to prevent, reduce or reverse the accumulation of α-synuclein-containing aggregates in tissue of a human subject diagnosed as having, or being at risk of developing, Lewy Body Dementia.

In one embodiment, said tissue is a neuron of the substantia nigra, cerebral cortex, ax, hippocampus, frontal lobes and/or temporal lobes.

In one embodiment, said dosage form comprises a further agent which is capable of treating or preventing said proteinopathy.

In a yet further aspect, the invention provides a pharmaceutical composition comprising:
(i) a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined in herein; and
(ii) a further agent which is capable of treating or preventing a proteinopathy; and
(iii) a pharmaceutically acceptable excipient.

In an embodiment, said further agent is selected from a dopamine precursor (e.g., L-DOPA), a dopamine agonist (e.g., bromocriptine, cabergoline, pergolide, pramipexole or apomorphine), a MAO-B inhibitor (e.g., rasagiline or selegiline), an anticholinergic (e.g., orphenadrine, procyclidine, or trihexyphenidyl), an enhancer of α-glucocerebrosidase activity (e.g., ambroxol or afegostat) and amantadine.

In another embodiment, said further agent is an acetylcholinesterase inhibitor (e.g., tacrine, rivastigmine, galantamine, donepezil, or memantine).

In an embodiment, the proteinopathy is a tauopathy selected from Parkinson's disease, Alzheimer's disease, Lewy Body Dementia, Pick's disease, progressive supranuclear palsy, dementia pugilistica, parkinsonism linked to chromosome 17, Lytico-Bodig disease, tangle-predominant dementia, Argyrophilic grain disease, ganglioglioma, gangliocytoma, meningioangiomatosis, subacute sclerosing panencephalitis, lead...
encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, lipofuscinosis, corticobasal degeneration, frontotemporal dementia, frontotemporal lobar degeneration and Huntington’s disease. In one embodiment, said tauopathy is Parkinson’s disease.

In another embodiment, the proteinopathy is a synucleinopathy selected from Lewy Body Dementia, Parkinson’s disease and multiple system atrophy.

In one embodiment, said composition is formulated for systemic administration, e.g. via a non-parenteral route. In one embodiment, said composition is formulated for oral administration.

In a related aspect, the invention provides a pharmaceutical dosage form, or a pharmaceutical composition, of the invention for use in therapy. In one embodiment, the pharmaceutical dosage form, or the pharmaceutical composition, is for use in a method as defined herein.

Additional features and advantages of compounds, compositions and methods disclosed herein will be apparent from the following detailed description.

**BRIEF DESCRIPTION OF THE DRAWINGS**

**FIG. 1** shows the results of a novel object recognition test carried out on mice, both wild-type (WT) and also in a proteinopathy model (Gba1<sup>D409V/D409V</sup>). Solid bars show target investigations during training and hatched bars show target investigations during testing. White bars (left of the figure) show results in WT mice, dark grey bars (middle of the figure) show results in untreated Gba1<sup>D409V/D409V</sup> mice, and light grey bars (right of the figure) show results in Gba1<sup>D409V/D409V</sup> mice treated with Compound 1.

**FIG. 2** shows the results of fear conditioning tests carried out on mice, both wild-type and also in a proteinopathy model (Gba1<sup>D409V/D409V</sup>). Figure 2A shows results relating to contextual memory. Figure 2B shows results relating to cued memory. White bars (left of the figures) show results in WT mice; hatched bars (middle of the figures) show results in untreated Gba1<sup>D409V/D409V</sup> mice, and black bars (right of the figures) show results in Gba1<sup>D409V/D409V</sup> mice treated with Compound 1.
FIG. 3 shows the results of target investigations during testing in a novel object recognition test carried out on mice, both wild-type (WT) and also in a proteinopathy model overexpressing Gba1 D409V α-synuclein (training results not shown). White bars (left of the figure) show results in WT mice, hatched bars (middle of the figure) show results in untreated Gba1 D409V/D409V mice treated with Compound 1, and black bars (right of the figure) show results in A53T mice treated with Compound 1.

FIG. 4 shows the results of fear conditioning tests carried out on mice, both wild-type (WT) and also in a proteinopathy model (overexpressing Gba1 D409V α-synuclein). FIG. 4A shows results relating to contextual memory. FIG. 4B shows the results relating to cued memory. White bars (left of the figures) show results in WT mice, hatched bars (middle of the figures) show results in untreated A53T mice, and black bars (right of the figures) show results in A53T mice treated with Compound 1.

FIG. 5 shows hippocampal quantification of ubiquitin aggregates in both wild-type and Gba1 D409V/D409V mice. FIG. 5A shows results at 16 weeks and FIG. 5B shows results at 40 weeks. White bars (far left of the figures) show results in WT mice, solid bars (second and left of the figures) show baseline levels in untreated Gba1 D409V/D409V mice at 4 weeks, and hatched bars (second right of the figures) show results in untreated Gba1 D409V/D409V mice, control mice, and black bars (far right of the figures) show results in Gba1 D409V/D409V mice treated with Compound 1.

FIG. 6 shows ubiquitin immunoreactivity (green) in the hippocampi of 40-week-old Gba1 D409V/D409V mice, either control (Fig. 6A) or treated with Compound 1 (Fig. 6B). DAPI nuclear staining is shown in blue.

FIG. 7 shows hippocampal quantification of proteinase K-resistant α-synuclein aggregates in both wild-type and Gba1 D409V/D409V mice. FIG. 7A shows results at 16 weeks and FIG. 7B shows results at 40 weeks. Striped bars (far left of the figures) show OW results in WT mice, white bars (second left of the figures) show baseline levels in untreated Gba1 D409V/D409V mice at 4 weeks, hatched bars (second right of the figures) show OW results in untreated Gba1 D409V/D409V mice, and black bars (far right of the figures) show OW results in Gba1 D409V/D409V mice treated with Compound 1.
FIG. 8 shows proteinase K-resistant α-synuclein immunoreactivity (red) in the hippocampi of 40-week-old Gba<sup>D409V/D409V</sup> mice, either control (Fig. 8A) or treated with Compound 1 (Fig. 8B). DAPI nuclear staining is shown in blue.

FIG. 9 shows hippocampal quantification of protein tau aggregates in both wild-type and D409V/D409V mice. Figure 9A shows results at 16 weeks and Figure 9B shows results at 40 weeks. White bars (far left of the figures) show results in WT mice, solid bars (second from left of the figures) show baseline levels in untreated Gba<sup>D409V/D409V</sup> mice at 4 weeks, ks, hatched bars (second from right of the figures) show results in untreated Gba<sup>D409V/D409V</sup> mice, ce, and black bars (far right of the figures) show results in Gba<sup>D409V/D409V</sup> mice treated with Compound 1.

FIG. 10 shows protein tau immunoreactivity (green) in the hippocampi of 40-week-old wild-type and D409V/D409V mice, either control (Fig. 10A) or treated with Compound 1 (Fig. 10B). DAPI nuclear staining is shown in blue.

FIG. 11 shows the subcellular localization of α-synuclein in cortical tissue homogenates from A53T mice, at 8 months of age. The levels of cytosolic soluble α-synuclein (Fig. 11A, A), membrane-associated (Fig. 11B), and cytosolic insoluble α-synuclein (Fig. 11C) are shown in untreated (left-hand black bar) and treated (right-hand grey bar) mice, ce.

FIG. 12 shows hippocampal quantification of ubiquitin aggregates in both wild-type and D409V/D409V A53T mice, at 8 months of age. The white bar (far left of the figure) shows results in WT T<sup>3T</sup> mice, the solid bar (second from left of the figure) shows baseline levels in untreated A53T T<sup>3T</sup> mice, at 6 weeks of age, the black bar (second from right of the figure) shows results in untreated A53T mice, and the grey bar (far right of the figure) shows results in A53T T<sup>3T</sup> mice treated with Compound 1.

FIG. 13 shows ubiquitin immunoreactivity (green) in the hippocampi of 8-month-old A53T mice, either control (Fig. 13A) or treated with Compound 1 (Fig. 13B). DAPI nuclear staining is shown in blue.

FIG. 14 shows hippocampal quantification of protein tau aggregates in both wild-type and D409V/D409V A53T mice, at 8 months of age. The white bar (far left of the figure) shows results in WT mice, the solid bar (second from left of the figure) shows baseline levels in untreated A53T T<sup>3T</sup> mice, and the black bar (second from right of the figure) shows results in A53T T<sup>3T</sup> mice treated with Compound 1.
mice at 6 weeks of age, the black bar (second right of the figure) shows results in untreated A53T mice, and the grey bar (far right of the figure) shows results in A53T T3T mice treated with Compound 1.

FIG. 15 shows protein tau immunoreactivity (green) in the hippocampi of 8-month-old A53T mice, either control (Fig. 15A) or treated with Compound II (Fig. 15B). DAPI, PI nuclear staining is shown in blue.

FIG. 16 shows the results of target investigations during testing in a novel object recognition test carried out on mice, both wild-type (WT) also in the G1-1 mouse model Gba

Although specific embodiments of the present disclosure will now be described, it should be understood that such embodiments are but a small number of the many possible specific embodiments, which can represent applications of the principles of the present disclosure. Various changes and modifications will be obvious to those of skill in the art, given the benefit of the present disclosure, and are deemed to be within the spirit and scope of the present disclosure as further defined in the appended claims.

Definitions:

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, exemplary methods, devices, and materials are now described. All technical and patent publications cited herein are incorporated herein by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

All numerical designations, e.g., pH, temperature, time, concentration, molecular weight, etc., are approximations, which are varied (±) or (10) by increments of 0.1 or 1.0, where appropriate. It is understood, although not always explicitly stated, that all numerical designations are preceded by the term "about." It also is to be understood, although not always explicitly stated, that the reagents described herein are merely exemplary, and that equivalents of such are known in the art.
term “including” is used herein to mean, and is used interchangeably with, the phrase “including but not limited to”. 

As used herein, the term “comprising” or “comprises” is intended to mean that the compositions and methods include the recited elements, but not excluding others. Thus, a composition consisting essentially of the elements as defined herein would not exclude trace contaminants from the isolation and purification method and/or pharmaceutically acceptable carriers, such as phosphate buffered saline, preservatives and the like. “Consisting of” shall mean excluding more than trace elements of other ingredients and substantial method steps for administering the compositions of this invention or process steps to produce a composition or achieve an intended result. 

Embodiments defined by each of these transition terms are within the scope of this invention. Use of the term “including” herein is intended to encompass “consisting of”.

The term, “proteinopathy” refers to a disease in which certain proteins become structurally abnormal and/or accumulate in a toxic manner, and thereby disrupt the function of cells, tissues, and organs of the body. Often the proteins fail to fold into their normal conformation. In this misfolded state, the proteins can become toxic or can lose their normal function. Non-limiting examples of proteinopathies include Alzheimer’s disease, senile dementia, progressive supranuclear palsy, dementia pugilistica, ataxia, Parkinsonism, Parkinson’s disease, dementia with Lewy bodies (also known as Lewy body disease), Pick’s disease, corticobasal degeneration, argyrophilic grain disease, subacute panencephalitis, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, and lipofuscinosis, cerebral atrophy, angiolipopathy, retinal ganglion cell degeneration in glaucoma, prion diseases, type 2 diabetes, amyotrophic lateral sclerosis (ALS), Huntington’s disease and other triplet repeat disorders, Alexander disease, seipinopathies, amyloidotic neuropathy, senile systemic amyloidosis, serpinopathies, amyloloidosis, inclusion body myositis/myopathy, Mallory bodies, pulmonary alveolar proteinosis, and critical illness myopathy (CIM).
As used herein, the term "chaperone" refers to a molecule, such as a small molecule, polypeptide, nucleic acid, and the like that specifically hinders to a protein (which is aberrant in a proteinopathy). The chaperone may restore or enhance at least partial wild-type function and/or activity of the protein (see e.g. Patnaik et al. (2012) J. Med. Chem. 55:5734-5748).

A "subject," "individual" or "patient" is used interchangeably herein, and refers to a vertebrate, such as a mammal. Mammals include, but are not limited to, murines, rats, rabbit, simians, bovines, ovine, porcine, canines, felines, farm animals, sport animals, pets, equines, primates, and humans. In one embodiment, the mammals include horses, dogs, and cats. In one embodiment, the mammal is a human.

"Administering" is defined herein as a means of providing an agent or a composition containing the agent to a subject in a manner that results in the agent being inside the subject's body. Such an administration can be by any route including, without limitation, oral, transdermal (e.g. vagina, rectum, oral mucosa), by injection (e.g. subcutaneous, intravenous, parenterally, intraperitonealy, into the CNS), or by inhalation (e.g. oral or nasal). Pharmaceutical preparations are, of course, given by forms suitable for each administration route.

"Treating" or "treatment" of a disease includes: (1) preventing the disease, i.e. causing the clinical symptoms of the disease not to develop in a patient that may be predisposed to the disease but does not yet experience or display symptoms of the disease; (2) inhibiting the disease, i.e. arresting or reducing the development of the disease or its clinical symptoms; and/or (3) relieving the disease, i.e. causing regression of the disease or its clinical symptoms.

The term "suffering" as it relates to the term "treatment" refers to a patient or individual who has been diagnosed with or is predisposed to the disease. A patient may also be referred to being "at risk of suffering" from a disease because of a history of disease in their family lineage or because of the presence of genetic mutations associated with the disease. A patient at risk of a disease has not yet developed all or some of the characteristic pathologies of the disease.
An "effective amount" or "therapeutically effective amount" is an amount sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages. Such delivery is dependent on a number of variables including the time period for which the individual dosage unit is to be used, the bioavailability of the therapeutic agent, the route of administration, etc. It is understood, however, that specific dose levels of the therapeutic agents of the present invention for any particular subject depends upon a variety of factors including, for example, the activity of the specific compound employed, the age, body weight, general health, sex, ex, and diet of the subject, the time of administration, the rate of excretion, the drug's combination, and the severity of the particular disorder being treated and form of administration. Treatment dosages generally may be titrated to optimize safety and efficacy. Typically, dosage-effect relationships from in vitro and/or in vivo tests initially can provide useful guidance on the proper doses for patient administration. In general, one will desire to administer an amount of the compound that is effective to achieve a serum level commensurate with the concentrations found to be effective in vitro.

Determination of these parameters is well within the skill of the art. These considerations, as well as effective formulations and administration procedures are well known in the art and are described in standard textbooks. Consistent with this definition, as used herein, in the term "therapeutically effective amount" is an amount sufficient to treat (e.g., improve) one or more symptoms associated with a proteinopathy or with aberrant/increased levels of α-synuclein, tau, or other protein aggregates in vivo, in vitro or in vivo. As used herein, the term "pharmaceutically acceptable excipient" encompasses any of the standard pharmaceutical excipients, including carriers such as a phosphate buffered saline solution, water, and emulsions, such as an oil/water or water/oil emulsion, and various US types of wetting agents. Pharmaceutical compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see Remington's Pharmaceuticals (20th ed., Mack Publishing Co., 2000).

As used herein, the term "prodrug" means a pharmacological derivative of a parent drug molecule that requires, biotransformation, either spontaneous or enzymatic, within the organism to release the active drug. For example, prodrugs are variations or derivatives of the quinuclidine compounds described herein that have groups cleavable under certain
metabolic conditions, which when cleaved, become the quinuclidine compounds described herein, e.g. a compound of Formula I. Such prodrugs then are pharmaceutically active in vivo when they undergo solvolysis under physiological conditions or undergo enzymatic degradation. Prodrug compounds herein may be called single, double, triple, etc., depending on the number of biotransformation steps required to release the active drug within the organism, and the number of functionalities present in a precursor-type prodrug form. Prodrug forms often offer advantages of solubility, tissue compatibility, or delayed release in the mammalian organism (Bundgard, Design of Prodrugs, pp. 7-9, 21-24, Elsevier, Amsterdam 1985 and Silverman, “The Organic Chemistry of Drug Design and Drug Action” pp. 352-401, Academic Press, San Diego, Calif., 1992).

Prodrugs commonly known in the art include well-known acid derivatives, such as, for example, esters prepared by reaction of acid compounds with a suitable alcohol, amides prepared by reaction of acid compounds with an amine, basic groups reacted to form an acetylated base derivative, etc. Other prodrug derivatives may be combined with other features disclosed herein to enhance bioavailability. As such, those of skill in the art will appreciate that certain of the presently disclosed compounds having, for example, free or hydroxy groups can be converted into prodrugs. Prodrugs include compounds having an amino acid residue, or a polypeptide chain of two or more (e.g., two, three, or four) amino acid residues, which are covalently joined through peptide bonds to free or amino, hydroxy, or carboxylic acid groups of the presently disclosed compounds. The amino, acid residues include all 20 naturally occurring amino acids commonly designated by three-letter symbols and also include 4-hydroxyproline, hydroxylysine, homoserine, isodemosine, 3-methylhistidine, norvalin, beta-alanine, gamma-amino butyric acid, citrulline, homocysteine, homoserine, ornithine, and methionine sulfone. Prodrugs also include compounds having a carbonate, carbamate, amide or alkyl ester moiety covalently bonded to any of the above substituents disclosed herein.

As used herein, the term “pharmaceutically acceptable salt” means a pharmaceutically acceptable acid addition salt or a pharmaceutically acceptable base addition salt of a a currently disclosed compound that may be administered without any resultant undesirable biological effect(s), or any resultant deleterious interaction(s) with any other component of a pharmaceutical composition in which it may be contained.
As used herein, the term “C\textsubscript{1-6}-alkyl” means a saturated linear or branched free radical consisting essentially of 1 to 6 carbon atoms and a corresponding number of hydrogen atoms. Exemplary C\textsubscript{1-6}-alkyl groups include methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, etc. Other C\textsubscript{1-6}-alkyl groups will be readily apparent to those of skill in the art given the benefit of the present disclosure. The terms “C\textsubscript{1-4}-alkyl”, “C\textsubscript{2-6}-alkenyl”, etc., have equivalent meanings, i.e., saturated linear or branched free radical consisting essentially of 1 to 3 (or 4) carbon atoms and a corresponding number of hydrogen atoms.

As used herein, the term “C\textsubscript{2-6}-alkenyl” means an unsaturated linear or branched free radical consisting essentially of 2 to 6 carbon atoms and a corresponding number of hydrogen atoms, which free radical comprises at least one carbon-carbon double bond. and.

Exemplary C\textsubscript{2-6}-alkenyl groups include ethenyl, prop-1-enyl, prop-2-enyl, isopropenyl, but-1-enyl, 2-methyl-prop-1-enyl, 2-methyl-prop-2-enyl, etc. Other C\textsubscript{2-6}-alkenyl groups will be readily apparent to those of skill in the art given the benefit of the present disclosure.

As used herein, the term “C\textsubscript{2-6}-alkynyl” means an unsaturated linear or branched free radical consisting essentially of 2 to 6 carbon atoms and a corresponding number of hydrogen atoms, which free radical comprises at least one carbon-carbon triple bond.

Exemplary C\textsubscript{2-6}-alkynyl groups include ethynyl, prop-1-ynyl, prop-2-ynyl, but-1-ynyl, 3-methyl-but-1-ynyl, etc. Other C\textsubscript{2-6}-alkynyl groups will be readily apparent to those of skill in the art given the benefit of the present disclosure.

As used herein, the term “C\textsubscript{1-6}-alkyloxy” means a saturated linear or branched free radical consisting essentially of 1 to 6 carbon atoms (and a corresponding number of hydrogen atoms) and an oxygen atom. Exemplary C\textsubscript{1-6}-alkyloxy groups include methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, etc. Other C\textsubscript{1-6}-alkyloxy groups will be readily apparent to those of skill in the art given the benefit of the present disclosure. The terms “C\textsubscript{1-4}-alkyloxy”, “C\textsubscript{2-6}-alkyloxy”, and the like, have an equivalent meaning, i.e., a saturated linear or branched free radical consisting essentially of 1 to 3 (or 4) carbon atoms (and a corresponding number of hydrogen atoms) and an oxygen atom, wherein the group is attached via the oxygen atom.
As used herein, the term “C_{2-6} -alkenylxy” means an unsaturated linear or branched free radical consisting essentially of 2 to 6 carbon atoms (and an corresponding number of of hydrogen atoms) and an oxygen atom, which free radical comprises at least one carbon-carbon double bond. An exemplary C_{2-6} -alkenylxy group is ethenylxy; others will be readily apparent to those of skill in the art given the benefit of the present disclosure.

As used herein, the term “C_{2-6} -alkenylxy” means an unsaturated linear or branched free radical consisting essentially of 2 to 6 carbon atoms (and an corresponding number of of hydrogen atoms) and an oxygen atom, which free radical comprises at least one carbon-carbon triple bond. An exemplary C_{2-6} -alkenylxy group is attached via the oxygen atom. An exemplary C_{2-6} -alkenylxy group is ethenylxy; others will be readily apparent to those of skill in the art given the benefit of the present disclosure.

As used herein, the term “heteroaryl” means an aromatic free radical having 5 or 6 atoms in a ring, wherein 1 to 5 of the ring atoms are carbon and the remaining 1 to 5 ring atoms (i.e., hetero ring atoms) is selected independently from the group consisting of nitrogen, sulfur, and oxygen. Exemplary 5-membered heteroaryl groups include: furyl, thienc, thiazolyl (e.g., thiazol-2-yl), pyrazolyl, isothiazolyl, oxazolyl, isoxazolyl, pyrrolyl, triazolyl, imidazolyl, oxadiazolyl and thiadiazolyl. Exemplary 6-membered heteroaryl groups include: pyridyl, pyrimidyl, pyrazinyl, pyridazinyl, 1,2,4-triazinyl, benzoxazolyl, benzothiazolyl, benzisothiazolyl, benzisoxazolyl, benzimidazolyl, etc. Other heteroaryl groups will be readily apparent to those of skill in the art given the benefit of the present disclosure. In general, the heteroaryl group typically is attached to the main structure via a carbon atom. However, certain other atoms, e.g., hetero ring atoms, can be be attached to the main structure.

As used herein, the term “aryl” means an aromatic free radical having 5 or 6 atoms in a ring, wherein all of the ring atoms are carbon. An exemplary aryl group is benzyl.

As used herein, the term “aliphatic” means a non-aromatic compound containing carbon and hydrogen atoms, e.g., containing 1 to 9 carbon atoms. Aliphatic compounds may be be
straight-chained or branched, may contain one or more ring structures, and may contain one or more carbon-carbon double bonds (provided that the compound does not contain an unsaturated ring structure having aromatic character). Examples of aliphatic compounds include ethane, propylene, cyclobutane, cyclohexadiene, etc.

As used herein, the terms “halo” and “halogen” mean fluorine, chlorine, bromine, or iodine. These terms are used interchangeably and may refer to a halogen free radical group or to a halogen atom as such. Those of skill in the art will readily be able to ascertain the identification of which in view of the context in which this term is used in the present disclosure.

As used herein, the term “cyano” means a free radical having a carbon atom linked to a nitrogen atom via a triple bond. The cyano radical is attached via its carbon atom.

As used herein, the term “nitro” means an NO₂ radical which is attached via its nitrogen atom.

As used herein, the terms “hydroxy” and “hydroxyl” mean an OH radical which is attached via its oxygen atom. The term “thio” means an SH radical which is attached via its sulfur atom.

As used herein, the term “amino” means a free radical having a nitrogen atom and 1 or 2 hydrogen atoms. As such, the term “amino” generally refers to primary and secondary amines. In that regard, as used herein and in the appended claims, a tertiary amine is represented by the general formula RR’N-, wherein R and R’ are carbon radicals that may be the same or different. Nevertheless, the term “amino” is generally used herein to describe a primary, secondary, or tertiary amine, and those of skill in the art will readily be able to ascertain the identification of, which in view of the context in which this term is used in the present disclosure.

As used herein, the term “oxo” means an oxygen radical which is attached via a a carbon-oxygen double bond. Where an atom bonded to this oxygen is a carbon atom, the bond is denoted (C=O) and, which may be referred to as a ketone.
The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

The following abbreviations are used herein:

- **A53T**: Transgenic mice expressing human α-synuclein with A53T mutation
- **ALS**: Amyotrophic lateral sclerosis
- **AMTS**: Abbreviated mental test score
- **ANOVA**: Analysis of variance
- **br**: Broad signal
- **CDI**: Carbonyldiimidazole
- **CIM**: Critical Illness Myopathy
- **CNS**: Central Nervous System
- **CS**: Conditioned stimulus
- **d**: Doublet
- **DAPI**: 4',6-diamidino-2-phenylindole
- **dd**: Doublet of doublets
- **DME**: Dimethoxyethane
- **DMSO-d6**: Dimethyl sulfoxide-d6
- **DMF**: Dimethylformamide
- **DNA**: Deoxyribonucleic acid
- **DTBZ**: Carbon-11 dihydrotetrabenazine
- **EDTA**: Ethylenediaminetetraacetic acid
- **ELISA**: Enzyme-linked immunosorbent assay
- **Et2O**: Diethyl ether
- **EtMgBr**: Ethylmagnesium bromide
- **EtOAc**: Ethyl acetate
- **FC**: Fear conditioning (test)
GBA1 Glucocerebrosidase 1 gene
HPLC: High pressure/performance liquid chromatography
HSA: Human serum albumin
IQCODE: Informant questionnaire on cognitive decline in the elderly
IPA: Isopropyl alcohol
ITI: Inter-trial interval
J: Coupling constant
LCMS: Liquid chromatography/mass spectrometry
m: Multiplet
MAPT: Microtubule-associated protein tau gene
MAO-B: Monoamine oxidase B
MMSE: Mini mental state examination
NOR: Novel object recognition (test)
PET: Positron emission tomography
PIB: Carbon-11 Pittsburgh Compound B
ppm: Parts per million
pTau: Phosphorylated Tau protein
rHA: Recombinant human albumin
s: Singlet
SNCA: α-synuclein gene
SPECT: Single-photon emission computed tomography
SEM: Standard error of mean
TBME: Tert-Butyl Methyl Ether
THF: Tetrahydrofuran
Tris: Tris(hydroxymethyl)aminomethane
TWEEN20: Polysorbate 20
TWEEN80: Polysorbate 80
WT: Wild type
UPLCMS: Ultra performance liquid chromatography/mass spectrometry
US: Unconditioned stimulus
US-CS: Unconditioned stimulus–Conditioned stimulus
Compounds

The present invention relates to quinuclidine compounds and their use in therapeutic methods relating to proteinopathies. In one aspect, the quinuclidine compound is a compound of formula (I),

\[
\begin{align*}
R^1 & \text{ is hydrogen; } \\
& \text{ or a pharmaceutically acceptable salt or prodrug thereof, wherein: } n:
\end{align*}
\]

\[
R^1 \text{ is hydrogen; or a halogen, or a C}_1-4\text{-alkyl, or C}_1-4\text{-alkyloxy group, optionally substituted by one or two groups selected independently from a halogen; }
\]

\[
R^2 \text{ and } R^3 \text{ are each independently selected from a C}_1-4\text{-alkyl group, optionally substituted by one or more (e.g., 1, 2 or 3) halogens; or a }
\]

\[
R^2 \text{ and } R^3 \text{ together form a cyclopropyl or cyclobutyl group, optionally substituted by one or more (e.g., 1 or 2) halogens; }
\]

\[
R^4, R^5 \text{ and } R^6 \text{ are each independently selected from hydrogen, a halogen, a nitro, hydroxy, thio, or amino group; and a C}_1-4\text{-alkyl or C}_1-4\text{-alkyloxy group, optionally substituted by one or more (e.g., 1 or 2) halogens; a }
\]

\[
\text{hydroxy or cyano group; and a C}_1-4\text{-alkyloxy group, and a }
\]

\[
A \text{ is a 5- or 6-membered aryl or heteroaryl group. }
\]

In one embodiment, R^1 is hydrogen; a halogen; or a C}_1-4\text{-alkyl or C}_1-4\text{-alkyloxy group, optionally substituted by one or two groups selected independently from a halogen; and a }

\[-27-\]
cyano, nitro, hydroxy, thio or amino group. In another embodiment, \( R' \) is hydrogen; or a methyl or ethyl group optionally substituted by a halogen, or a hydroxy, thio or amino group. In a further embodiment, \( R' \) is hydrogen; or a methyl group optionally substituted by one or more \((e.g., 1, 2 \text{ or } 3)\) halogens. In a yet further embodiment, \( R' \) is hydrogen. In one embodiment, \( R' \) is not attached to the nitrogen atom of the quinuclidine moiety.

In one embodiment, \( R'' \) and \( R' \) are each independently selected from \( C_{1-3} \)-alkyl groups, optionally substituted with one or more halogens. In another embodiment, \( R'' \) and \( R' \) are each independently selected from methyl and ethyl groups, optionally substituted with one or more fluorine atoms. In a further embodiment, \( R'' \) and \( R' \) are each methyl, \( y \) or \( 1 \), systematically selected from fluorine, optionally substituted with one or more \((e.g., 1, 2 \text{ or } 3)\) halogens, and a cyano or \( C_{1-3} \)-alkyl group. In another embodiment, \( R'' \) is selected from a halogen; and \( y \) cyano or \( C_{1-3} \)-alkyl group. In another embodiment, \( R'' \) is selected from a halogen; and \( y \) cyano or \( C_{1-3} \)-alkyl group, optionally substituted by one or more groups selected from a halogen, and a \( C_{1-3} \)-alkyl group. In another embodiment, \( R'' \) is selected from a halogen; and \( y \) cyano or \( C_{1-3} \)-alkyl group, optionally substituted by one or more \((e.g., 1, 2 \text{ or } 3)\) halogens, and a \( C_{1-3} \)-alkyl group. In a further embodiment, \( R'' \) is selected from fluorine; and a \( C_{1-3} \)-alkyl group, optionally substituted by one or more groups selected from a halogen, and a cyano or \( C_{1-3} \)-alkyl group. In another embodiment, \( R'' \) and \( R' \) are both methyl groups. For example, where \( R' \) and \( R' \) are both methyl hydrogens, \( R'' \) may be fluorine or a 2-methoxyethoxy group, \( e.g., \) fluorine or.

Where all of \( R' \), \( R'' \) and \( R' \) are other than hydrogen, these three groups may be attached to the benzene ring, for example, at positions 2, 4 and 6 (relative to the group \( A \) being attached to position 1). Where only one of \( R' \), \( R'' \) and \( R' \) is hydrogen, the other two are groups which may be attached to the benzene ring, for example, at positions 2 and 3, positions 3 and 4, or positions 3 and 5, \( e.g., \) at positions 3 and 5 (relative to the group \( A \) being
attached to position 1). Where two of \( R^1, R^2 \) and \( R^3 \) are hydrogen, the other group may be attached to the benzene ring at position 2, 3 or 4, e.g. at position 4 (i.e. at the \textit{para} to the group A). In one embodiment, \( R^1 \) is in a position on the benzene ring \textit{para} to the group A.

In one embodiment, \( A \) is a 6-membered aryl group or a 5-membered heteroaryl group. Non-limiting examples of 6-membered aryl groups and 5-membered heteroaryl groups include benzyl, furyl, thienyl, thiazolyl, pyrazolyl, isothiazolyl, oxazolyl, isoxazolyl, yl, pyrrolyl, triazolyl, imidazolyl, oxadiazolyl and thiadiazolyl. In one embodiment, the 6-membered aryl group or 5-membered heteroaryl group is selected from benzyl, thienyl, yl, thiazolyl, pyrrolyl and imidazolyl. In another embodiment, the 6-membered aryl group or 5-membered heteroaryl group is selected from benzyl and thiazolyl yl.

In one embodiment, \( A \) is benzyl, optionally substituted with 1, 2 or 3 groups independently selected from a halogen, hydroxy, amino, nitro, ester, or methyl group. In another embodiment, \( A \) is benzyl, optionally substituted with 1 or 2 halogens. In a further embodiment, \( A \) is benzyl, optionally substituted with a halogen, e.g. fluorine. In a yet further embodiment, \( A \) is an unsubstituted benzyl group.

Where \( A \) is a 6-membered aryl or heteroaryl, the attached groups \( -C(R^1 R^2) \) and \( -(C_n H_n R^1 R^2) \) may be in a 1,2-, 1,3- or 1,4-relationship, i.e. ortho, meta or para to each other. In one embodiment, the attached groups \( -C(R^1 R^2) \) and \( -(C_n H_n R^1 R^2) \) remain in a 1,3-relationship. In another embodiment, the attached groups are in a 1,4-relationship.

In one embodiment, \( A \) is a 5-membered heteroaryl group which contains 1, 2 or 3 heteroatoms selected from N, O and S. In another embodiment, \( A \) is a 5-membered heteroaryl group which contains 1 or 2 heteroatoms selected from N and S. In a further embodiment, \( A \) is a 5-membered heteroaryl group which contains 2 heteroatoms selected from N and S. In a yet further embodiment, \( A \) is a 5-membered heteroaryl group which contains 2 heteroatoms wherein one heteroatom is N and the other heteroatom is S. In a still further embodiment, \( A \) is a thiazolyl group.

Where \( A \) is a 5-membered heteroaryl group, at least one of the attached groups \( -C(R^1 R^2) \) and \( -(C_n H_n R^1 R^2) \) may be bonded directly to a carbon atom of the heteroaryl group. In one embodiment, both of the attached groups \( -C(R^1 R^2) \) and \( -(C_n H_n R^1 R^2) \) are bonded
directly to a carbon atom of the heteroaryl group. In one embodiment, the attached groups are C(R₂R₃) and -(C₆H₂R₄R₅R₆) which are in a 1,3-relationship to each other, e.g., they are bonded directly to carbon atoms of the heteroaryl group which are separated by a single intervening atom, e.g., heteroatom. In another embodiment where A is a thiazolyl group, the attached groups are C(R₂R₃) and -(C₆H₂R₄R₅R₆) may be bonded directly at the 4- and 2-positions, respectively.

Thus, in one embodiment the quinuclidine compound is a compound of formula (II)

![Diagram of compound II]

(II)

or a pharmaceutically acceptable salt or prodrug thereof, wherein R', R', R' and A are as defined herein.

In another embodiment, the quinuclidine compound is a compound of formula (III)

![Diagram of compound III]

(III)

or a pharmaceutically acceptable salt or prodrug thereof, wherein R', R' to R', and A are as defined herein.

In another embodiment, the quinuclidine compound is a compound of formula (IV)

- 30 -
or a pharmaceutically acceptable salt or prodrug thereof, wherein $R^4$ and $A$ are as defined herein.

In one embodiment, $R^4$ is a halogen, e.g. fluorine. Accordingly, the quinuclidine compound may be a compound of formula (V)

or a pharmaceutically acceptable salt or prodrug thereof, wherein $A$ is as defined herein.

In another embodiment, the quinuclidine compound is a compound of formula (VI)

or a pharmaceutically acceptable salt or prodrug thereof, wherein $R^1$ to $R^6$ are as defined herein.
In another embodiment, the quinuclidine compound is a compound of formula (VII) or a pharmaceutically acceptable salt or prodrug thereof, wherein R¹ to R⁶ are defined herein.

(VII)

or a pharmaceutically acceptable salt or prodrug thereof, wherein R¹ to R⁶ are defined herein.

In another embodiment, the quinuclidine compound is a compound of formula (VIII) or

(VIII)

or a pharmaceutically acceptable salt or prodrug thereof, wherein R¹ to R⁶ are defined herein.

In another embodiment, the quinuclidine compound is a compound of formula (IX) or

(IX)
or a pharmaceutically acceptable salt or prodrug thereof, wherein $R^4$ is as defined herein.

In one embodiment, $R^4$ is a halogen, e.g., fluorine. Accordingly, the quinuclidine compound may be a compound of formula (X).

or a pharmaceutically acceptable salt or prodrug thereof, wherein $R^4$ is as defined herein.

In another embodiment, the quinuclidine compound is a compound of formula (XI).

or a pharmaceutically acceptable salt or prodrug thereof, wherein $R^4$ is as defined herein.
In one embodiment, $R^4$ is a halogen, e.g., fluorine. Accordingly, the quinuclidine compound may be a compound of formula (XII):

![Chemical Structure](image)

(XII)

or a pharmaceutically acceptable salt or prodrug thereof.

In one embodiment, the quinuclidine compound is selected from the group consisting of:

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Compound</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Quinuclidin-3-yl (2-(4'-fluoro-[1,1'-biphenyl]-3-yl)propan-2-yl)carbamate</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>(S)-quinuclidin-3-yl (2-(2-(4-fluorophenyl)thiazol-4-y1)propan-2-yl)carbamate</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>(S)-quinuclidin-3-yl (2-(4'-(2-methoxyethoxy)-[1,1'-biphenyl]-4-yl)propan-2-yl)carbamate</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1-azabicyclo[2.2.2]oct-3-yl [2-(biphenyl-3-yl)propan-2-yl]carbamate</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>(S)-quinuclidin-3-yl 2-(biphenyl-4-yl)propan-2-ylcarbamate</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Quinuclidin-3-yl 1-(biphenyl-4-yl)cyclopropylcarbamate</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>(S)-quinuclidin-3-yl 1-(4'-fluorobiphenyl-4-yl)cyclopropylcarbamate</td>
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</tr>
<tr>
<td>8</td>
<td>(S)-1-azabicyclo[2.2.2]oct-3-yl [1-(2',4'-difluorobiphenyl-4-yl)cyclopropyl]carbamate</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1-azabicyclo[2.2.2]oct-3-yl [1-(4'-methoxybiphenyl-4-yl)cyclopropyl]carbamate</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Quinuclidin-3-yl 2-(5-(4-fluorophenyl)thiophen-3-yl)propan-2-ylcarbamate</td>
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</tr>
<tr>
<td>Compound No.</td>
<td>Compound</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>--------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>(S)-quinuclidin-3-yl 2-(3-(4-fluorophenyl)isothiazol-5-yl)propan-2-ylcarbamate</td>
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<tr>
<td>12</td>
<td>(S)-quinuclidin-3-yl 2-(4-(4-fluorophenyl)thiazol-2-yl)propan-2-ylcarbamate</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Quinuclidin-3-yl (2-(4’-(2-methoxyethoxy)-[1,1’-biphenyl]-4-yl)propan-2-yl)carbamate</td>
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<td>14</td>
<td>(S)-quinuclidin-3-yl (2-(3’-(2-methoxyethoxy)-[1,1’-biphenyl]-4-yl)propan-2-yl)carbamate</td>
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</tr>
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<td>Quinuclidin-3-yl (2-(4’-(2-hydroxyethyl)-[1,1’-biphenyl]-4-yl)propan-2-yl)carbamate</td>
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<tr>
<td>19</td>
<td>Quinuclidin-3-yl (2-(2-(4-(5-methoxypropoxy)phenyl)isothiazol-4-yl)propan-2-yl)carbamate</td>
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<tr>
<td>21</td>
<td>Quinuclidin-3-yl 2-(5-(4-(2-methoxyethoxy)phenyl)pyridin-2-yl)propan-2-yl)carbamate</td>
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<tr>
<td>22</td>
<td>Quinuclidin-3-yl (2-(4’-(3-cyanoproxy)-[1,1’-biphenyl]-4-yl)propan-2-yl)carbamate</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>Quinuclidin-3-yl (2-(4’-(cyanomethoxy)-[1,1’-biphenyl]-4-yl)propan-2-yl)carbamate</td>
<td></td>
</tr>
</tbody>
</table>

and the pharmaceutically acceptable salts and prodrugs thereof.

In one embodiment, the quinuclidine compound is selected from Compound 1, Compound 2, or a pharmaceutically acceptable salt or prodrug thereof. In another embodiment, the quinuclidine compound is selected from Compound 1, Compound 2, or a pharmaceutically acceptable salt or prodrug thereof. In another embodiment, the quinuclidine compound is Compound 1, or a pharmaceutically acceptable salt thereof.
acceptable salt or prodrug thereof. In another embodiment, the quinuclidine compound is Compound 2, or a pharmaceutically acceptable salt or prodrug thereof. In another embodiment, the quinuclidine compound is Compound 3, or a pharmaceutically acceptable salt or prodrug thereof.

In another embodiment, the quinuclidine compound is selected from Compound 1, Compound 2, and Compound 3. In one embodiment, the quinuclidine compound is Compound 1. In another embodiment, the quinuclidine compound is Compound 2. In another embodiment, the quinuclidine compound is Compound 3.

Salts;

Presently disclosed compounds that are basic in nature are generally capable of forming a wide variety of different salts with various inorganic and/or organic acids. Although such salts are generally pharmaceutically acceptable for administration to animals and humans, it is often desirable in practice to initially isolate a compound from the reaction mixture as a pharmaceutically unacceptable salt and then simply convert the latter back to the free free base compound by treatment with an alkaline reagent, and subsequently convert the free free base to a pharmaceutically acceptable acid addition salt. The acid addition salts of the base compounds can be readily prepared using conventional techniques, e.g., by treating the base compound with a substantially equivalent amount of the chosen mineral or organic acid in an aqueous solvent medium or in a suitable organic solvent such as, for example, methanol or ethanol. Upon careful evaporation of the solvent, the desired solid, i.e., salt, is obtained. Presently disclosed compounds that are positively charged, e.g., g., containing a quaternary ammonium, may also form salts with the anionic component of a wide variety of inorganic and/or organic acids.

Acids, which can be used to prepare pharmaceutically acceptable salts of quinuclidine compounds, are those, which can form non-toxic acid addition salts, e.g., salts containing pharmacologically acceptable anions, such as chloride, bromide, iodide, nitrate, sulfate or bisulfate, phosphate, or an acid, phosphate, acetate, lactate, citrate, or acid citrate, tartrate, or bitartrate, succinate, malate, maleate, fumarate, gluconate, saccharate, benzoate, and methanesulfonate and pamoate [(i.e., 1,1’-methylene-bis-(2-hydroxy-3-naphthoate)] salts.  

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Presently disclosed compounds that are acidic in nature, e.g., compounds containing a tetrazole moiety, are generally capable of forming a wide variety of different salts with various inorganic and/or organic bases. Although such salts are generally pharmaceutically acceptable for administration to animals and humans, it is often desirable in practice to initially isolate a compound from the reaction mixture as a pharmaceutically unacceptable salt and then simply convert the latter back to the free acid or compound by treatment with an acidic reagent, and subsequently convert the free acid to a pharmaceutically acceptable base addition salt. These base addition salts can be readily prepared using conventional techniques, e.g., by treating the corresponding acidic compounds with an aqueous solution containing the desired pharmaceutically acceptable base cations, and then evaporating the resulting solution to dryness, e.g., under reduced pressure. Alternatively, they also can be prepared by mixing lower alkanolic solutions of the acidic compounds and the desired alkaline metal alkoxide together, and then evaporating the resulting solution to dryness in the same manner as before. In either case, stoichiometric quantities of reagents may be employed in order to ensure completeness of the reaction, and maximum product yields of the desired solid salt is the case.

Bases, which can be used to prepare the pharmaceutically acceptable base addition salts of quinuclidine compounds, are those which can form non-toxic base addition salts, e.g., salts containing pharmaceutically acceptable cations, such as, alkali metal cations (e.g., potassium and sodium), alkaline earth metal cations (e.g., calcium and magnesium), ammonium, or other water-soluble amine addition salts such as N-methylglucamine (meglumine), lower alkanolic ammonium, and other such bases of organic amines, etc.

In one embodiment, the pharmaceutically acceptable salt is a succinate salt. In another embodiment, the pharmaceutically acceptable salt is a 2-hydroxysuccinate salt, e.g., an (S)-2-hydroxysuccinate salt. In another embodiment, the pharmaceutically acceptable salt is an hydrochloride salt (i.e., a salt with HCl). In another embodiment, the pharmaceutically acceptable salt is a malate salt.

Prodrugs

The pharmaceutically acceptable prodrugs disclosed herein are derivatives of quinuclidine compounds, which can be converted in vivo into the quinuclidine compounds.
described herein. The prodrugs, which may themselves have some activity, become pharmaceutically active in vivo when they undergo, for example, solvolysis under physiological conditions or enzymatic degradation. Methods for preparing prodrugs of compounds as described herein would be apparent to one of skill in the art based on the present disclosure.

In one embodiment, the carbamate moiety of the quinuclidine compound is modified. For example, the carbamate moiety of the quinuclidine compound may be modified by the addition of water and/or two or more aliphatic alcohols. In this case, the carbon-oxygen double bond of the carbamate moiety adopts what could be considered a hemiacetal or acetal functionality. In one embodiment, the carbamate moiety of the quinuclidine compound may be modified by the addition of an aliphatic diol such as 1,2-ethanediol.

In one embodiment, one or more of the hydroxy, thio or amino groups on the quinuclidine compound are modified. For example, one or more of the hydroxy, thio and/or amino groups on the quinuclidine compound may be modified to form acid derivatives, e.g., esters, thioesters (orthioesters) and/or amides. The acid derivatives can be formed, for example, by reacting a quinuclidine compound, which comprises one or more hydroxy, thio or amino groups, with an acetylating agent. Examples of acetylating agents include anhydrides such as acetic anhydride, acid chlorides such as benzyl chloride, and diesters such as tert-butyl dicarbonate.

Stereochemistry

Stereoisomers (e.g., cis and trans isomers) and all optical isomers of a presently disclosed compound (e.g., R- and S-enantiomers), as well as racemic, diastereomeric and other mixtures of such isomers are within the scope of the present disclosure.

In one embodiment, the quinuclidin-3-yl group of a quinuclidine compound as defined herein has the R-configuration. Accordingly, the quinuclidine compound may be selected from the group consisting of compounds of formulae (Ia) to (XIIa).
and the pharmaceutically acceptable salts and prodrugs thereof.

In another embodiment, the quinuclidin-3-yl group of the quinuclidine compound as defined herein has the S- configuration. Accordingly, the quinuclidine compound may be selected from the group consisting of compounds of formulae (Ib) to (XIIb):

![Compound Structures](image-url)
In one embodiment the quinuclidine compound is a compound of formula (Xb), or a pharmaceutically acceptable salt or prodrug thereof. In another embodiment the quinuclidine compound is a compound of formula (XIIb), or a pharmaceutically acceptable salt or prodrug thereof.

In one embodiment, the quinuclidine-3-yl group of the quinuclidine compound as defined herein, exists in a mixture of isomers having the R- and S-configurations. For example, the quinuclidine compound may be a mixture of compounds selected from the group consisting of compounds of formulae (Ia) and (Ib), (IIa) and (IIb), (IIIa) and (IIIb), (IVa), (Va) and (Vb), (VIa) and (VIb), (VIIa) and (VIIb), (VIIIa) and (VIIIb), (IXa) and (IXb), (Xa) and (Xb), and the pharmaceutically acceptable salts and prodrugs thereof. In one embodiment, the quinuclidine compound is present as a racemic mixture, e.g., the R- and S-isomers of the quinuclidine-3-yl group are present in about equal amounts. In another embodiment, the quinuclidine compound is present as a single enantiomer or diastereomer.
quinuclidine compound is present as a mixture of isomers having the R- and S-\( \rightarrow \) \( \rightarrow \) configurations. Wherein the R- and S- isomers are present in different amounts. In one embodiment the S- isomer is present in an enantiomeric excess of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 99.9%, e.g., about 100%. In another embodiment, the R- isomer is present in an enantiomeric excess of at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 99.9%, e.g., about 100%.%.

Methods for preparing enantiorenhanced and/or enantiopure quinuclidine compounds as would be apparent to the person of skill in the art based on the present disclosure. re.

The compounds presently disclosed can exist in several tautomeric forms, including the enol and imine forms, and the keto and enamine forms and geometric isomers and mixtures thereof. Tautomers exist as mixtures of tautomer set at equilibrium. In solid form, usually one tautomer predominates. Even though one tautomer may be described, all tautomers are within the scope of the present disclosure.

Atropisomers are also within the scope of the present disclosure. Atropisomers refer to compounds that can be separated into rotationally restricted isomers.

Other forms.

Pharmaceutically acceptable hydrates, solvates, polymorphs, etc., of the quinuclidine compounds described herein are within the scope of the present disclosure. Quinuclidine compounds as described herein may be in an amorphous form and/or in one or more crystalline forms.

Isotopically-labeled compounds are also within the scope of the present disclosure. As used herein, an "isotopically-labeled compound" refers to a presently disclosed, e.g., compound including pharmaceutical salts and prodrugs thereof, each as described herein, in which one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature, e.g., examples of isotopes that can be incorporated into compounds presently disclosed, e.g., include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, fluorine and chlorine, such as \( ^2H \), \( ^3H \), \( ^14C \), \( ^13C \), \( ^15N \), \( ^16O \), \( ^17O \), \( ^31P \), \( ^32P \), \( ^33P \), \( ^34S \), \( ^18F \) and \( ^35Cl \), respectively.
Medical indications

The quinuclidine compounds, and pharmaceutical compositions containing them, described herein are useful in therapy, in particular in the therapeutic treatment of proteinopathies in a subject. Subjects to be treated according to the methods described herein include vertebrates, such as mammals. In particular embodiments the mammal is a human patient.

The present invention provides a method for treating a proteinopathy in a subject, the method comprising administering to the subject an effective amount of a quinuclidine compound as described herein. Also provided is a quinuclidine compound as described herein for use in a method of treating a proteinopathy in a subject. Further provided is the use of a quinuclidine compound as described herein in the manufacture of a medicament for use in a method of treating a proteinopathy in a subject. In one embodiment, the subject is a human subject.

In one embodiment, the proteinopathy recited in the methods disclosed herein is a disease selected from the group consisting of Alzheimer’s disease, frontotemporal dementia, progressive supranuclear palsy, Parkinsonism, Parkinson’s disease, Lytico-Bodig disease, se, dementia with Lewy bodies, tangle-predominant dementia, dementia pugilistica, Pick’s disease, corticobasal degeneration, Argyrophilic grain disease, gangliogioma and gangliocytoma, meningioangiomatosis, subacute sclerosing panencephalitis, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, and lipofuscinosis. In one embodiment the proteinopathy is Alzheimer’s disease. In another embodiment the proteinopathy is dementia with Lewy bodies. In another embodiment the proteinopathy is Parkinson’s disease.

In one embodiment, the proteinopathy is a tauopathy. Tauopathies are neurodegenerative disorders characterized by accumulation of tau. Exemplary tauopathies include, for example, Alzheimer’s disease, progressive supranuclear palsy, dementia pugilistica, ca, Parkinson’s disease, parkinsonism linked to chromosome 17, Lytico-Bodig disease, se, tangle-predominant dementia, Argyrophilic grain disease, gangliogioma, gangliocytoma, na, meningioangiomatosis, subacute sclerosing panencephalitis, lead encephalopathy, ty, tuberous sclerosis, Hallervorden-Spatz disease, lipofuscinosis, dementia with Lewy bodies.
bodies, Pick’s disease, corticobasal degeneration, frontotemporal dementia, Alzheimer’s disease, Parkinson’s disease, multiple system atrophy, and Lewy Body dementia. Some diseases classified as synucleinopathies may also have accumulation of the tau protein, and some of these diseases classified as tauopathies may also have accumulation of the α-synuclein protein. Accordingly, in one embodiment the proteinopathy is characterized by the accumulation of α-synuclein and tau.

The methods disclosed herein are useful for treating subjects (e.g., mammals such as humans) with a proteinopathy. In certain embodiments, the proteinopathy involves α-synuclein aggregates. Protein “aggregation” refers to the biological phenomenon in which misfolded proteins aggregate either intra- or extra-cellularly. These protein aggregates may be toxic. In certain embodiments, the protein aggregates comprise a protein selected from the group consisting of ubiquitin, α-tau, and α-synuclein.

Ubiquitin is a small protein that is found in almost all tissues of eukaryotic organisms. It is a 76-amino acid protein that can be attached to a substrate protein. Addition of ubiquitin can result in protein degradation; modulation of transcription, translation, and protein localization; or modulation of protein activity/interactions. Tau proteins function to stabilize microtubules and α-tau protein can be found in different parts of the cell, such as in the membrane, soluble in the cytosol, and insoluble in the cytosol. They are abundant in neurons of the central nervous system and in astrocytes and oligodendrocytes.

Hyperphosphorylation of the tau protein (α-tau, inclusions, “pTau”) can result in the self-assembly of filaments of paired helical filaments and straight filaments, which are involved in the pathogenesis of Alzheimer’s disease and other tauopathies. All of the six tau isoforms are present in Alzheimer’s disease brain. In other neurodegenerative diseases, the deposition of aggregates enriched in certain tau isoforms has been reported. When misfolded, this otherwise very soluble protein can form extremely insoluble aggregates that contribute to a number of neurodegenerative diseases. α-Synuclein is a protein that, in humans, is encoded by the SNCA gene. α-Synuclein can be found in different parts of the cell, such as in the membrane, soluble in the cytosol, and insoluble in the cytosol. The protein is found in...
primarily in neural tissue and is predominantly expressed in the neocortex, hippocampus, US, substantia nigra, thalamus, and cerebellum. Besides neurons, the protein can also be found in neuroglial cells and melanocytic cells. α-synuclein can aggregate to form pathologically insoluble fibrils in pathological conditions that are, at some instances, characterized by Lewy bodies.

The present invention also provides a method of reducing, reversing or preventing the accumulation of protein aggregates in tissue of a subject diagnosed as having α-synucleinopathy, or diagnosed as being at risk of developing α-synucleinopathy. The method comprises administering to the subject an effective amount of a quinuclidine compound as described herein. In related aspects, the invention provides a quinuclidine compound as described herein for use in a method of reducing, reversing or preventing the accumulation of protein aggregates in tissue of a subject diagnosed as having α-synucleinopathy, or diagnosed as being at risk of developing α-synucleinopathy. In other related aspects, the invention provides the use of a quinuclidine compound as described herein in the manufacture of a medicament for use in a method of reducing, reversing or preventing the accumulation of protein aggregates in tissue of a subject diagnosed as having α-synucleinopathy, or diagnosed as being at risk of developing α-synucleinopathy.

In one embodiment, the protein aggregates comprise ubiquitin, protein tau and/or α-synuclein. In another embodiment, the protein aggregates comprise protein tau or α-synuclein. In one embodiment, the protein aggregates comprise protein tau and α-synuclein. In another embodiment, the subject does not have protein aggregates comprising α-synuclein in said tissue. In one embodiment, the subject does not have protein aggregates comprising α-synuclein in said tissue. In another embodiment, the protein aggregates are α-synuclein aggregates, comprising α-synuclein aggregates, and the proteinopathy is α-synucleinopathy. In another embodiment, the protein aggregates are α-synuclein aggregates, comprising α-synuclein aggregates, and the proteinopathy is a tauopathy, e.g. Parkinson’s disease.
of the substantia nigra, cerebral cortex, hippocampus, frontal lobes and/or temporal lobes.
In a further embodiment, the proteinopathy is Parkinson's disease characterised by the presence of protein tau, but not α-synuclein, within protein aggregates in the central nervous system of the subject, e.g., in neurons of the substantia nigra, cerebral cortex, ex, hippocampus, frontal lobes and/or temporal lobes of the subject.

In certain embodiments, the methods described herein are effective in reducing a specific fraction of α-synuclein. In one embodiment, cytosolic insoluble α-synuclein is reduced. In another embodiment, membrane-associated α-synuclein is reduced. In a further embodiment, extracellular α-synuclein is reduced. In embodiments, aggregated α-synuclein is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100%. In one embodiment, α-synuclein is reduced to a level not significantly different to that of a subject (e.g., a mammal such as a human) without a proteinopathy characterised by an increase in: in α-synuclein.

Administration of quinuclidine compounds as described herein to a subject can alter the the processing and localization of α-synuclein within the CNS of the subject, e.g., within in cortical tissue in the brain. In one embodiment of the methods described herein, levels of membrane-associated α-synuclein and/or insoluble cytosolic α-synuclein are reduced. In certain embodiments, the levels of membrane-associated α-synuclein and/or insoluble cytosolic α-synuclein are reduced, but levels of soluble cytosolic α-synuclein are not reduced (e.g., are not significantly altered). In particular embodiments, the subject is a human, subject diagnosed as having a synucleinopathy, or is diagnosed as being at risk of developing a synucleinopathy, especially Parkinson's Disease or Lewy Body Dementia.

In certain embodiments, the methods described herein are effective in reducing a specific fraction of tau. In one embodiment, cytosolic insoluble tau is reduced. In another embodiment, the membrane-associated tau is reduced. In a further embodiment, extracellular tau is reduced. In embodiments, aggregated tau is reduced by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, or at least about 30%.
Proteinopathies, especially when present in the central nervous system, can result in an impairment of neural function, e.g., cognitive function, autonomic function and/or motor function. Administration of quinuclidine compounds described herein can result in the improvement of neural function in subjects, e.g., in subjects exhibiting cognitive impairment due to a proteinopathy. Accordingly, in certain embodiments of the present methods, a quinuclidine compound described herein is administered to a subject having impaired neural (e.g., neurologic) function. In particular embodiments, administration of the quinuclidine compound is initiated after the subject has been diagnosed with impaired neural (e.g., neurologic) function. Diagnosis of a cognitive impairment is within the routine skill of a medical practitioner. Cognitive tests are known in the art and can include tests such as the abbreviated mental test score (AMTS), the mini mental state examination (MMSE), informant questionnaire on cognitive decline in the elderly (IQCODE), and the General Practitioner Assessment of Cognition (GAPAC). These tests can assess impairments in, for example, memory, reasoning skills, problem solving skills, decision-making skills, attention span, and language skills. Imaging methods are also available to diagnose cognitive decline. For example, the functional neuroimaging modalities of single photon emission computed tomography (SPECT) and positron emission tomography (PET) are useful in assessing cognitive dysfunction. In some cases, the improvement of neural function is measured by evaluating the cognitive function of the patient. Cognitive deterioration, e.g., associated with mild cognitive impairment, may also be assessed by monitoring different cognitive domains. Cognitive domains include, for example, attention and concentration, executive functions, memory, verbal language, visuo-constructional skills, conceptual thinking, calculations, and orientation. Diagnosis of other impairments associated with proteinopathies is also within the routine skill of a medical practitioner. For example, clinical criteria for a diagnosis of Parkinson’s disease involve assessing impairments in motor and/or autonomic functions, e.g., slowness.
of movement (bradykinesia) plus either rigidity, resting tremor, or postural instability.

Responsiveness to dopamine (symptomatic treatment) and reduced dopaminergic activity in the basal ganglia can also aid in diagnosing Parkinson’s disease.

In relation to methods for preventing cognitive decline, such as memory loss, PET imaging using carbon-11 Pittsburgh Compound B (PIB-PET) has been useful in predicting which patients with mild cognitive impairment would develop Alzheimer’s disease within two years. In another study, using either PIB or another radiotracer, carbon-11 dihydrotetabenazine (DTBZ), led to more accurate diagnosis of Alzheimer’s disease.

The methods described herein can prevent, reduce, or reverse loss of neural function in a subject diagnosed as having, or at risk of developing, a quinuclidine proteinopathy. Accordingly, the invention provides a method of preventing, reducing or reversing loss of neural function in a subject diagnosed as having, or at risk of developing, a quinuclidine proteinopathy. The method comprises administering to the subject an effective amount of a quinuclidine compound as described herein.

As a related aspect, the invention provides a quinuclidine compound as described herein for use in a method of preventing, reducing or reversing loss of neural function in a subject diagnosed as having, or at risk of, a proteinopathy. The amount of neural function may comprise loss of cognitive function, autonomic function and/or motor function.

The methods described herein can prevent, reduce, or reverse the progression of dementia. Accordingly, the invention provides a method of preventing, reducing or reversing the progression of dementia in a subject diagnosed as having, or at risk of developing, a quinuclidine proteinopathy. The method comprises administering to the subject an effective amount of a quinuclidine compound as described herein. In related aspects, the invention provides as a quinuclidine compound as described herein for use in a method of preventing, reducing or reversing the progression of dementia in a subject diagnosed as having, or at risk of, a quinuclidine proteinopathy.
developing, a proteinopathy. In other related aspects, the invention provides the use of a quinuclidine compound as described herein in the manufacture of a medicament for use in a method of preventing, reducing or reversing the progression of dementia in a subject diagnosed as having, or at risk of developing, a proteinopathy. Symptoms of dementia which may be prevented, reduced or reversed include early symptoms of dementia, such as difficulty remembering recent conversations, names or events, and apathy and depression, as well as later symptoms, such as impaired communication, poor judgment, disorientation, confusion, behavior changes and difficulty in speaking, swallowing and/or walking.

The methods described herein may also be used to prevent or treat cognitive impairment, e.g. mild cognitive impairment. Mild cognitive impairment is an intermediate stage between the expected cognitive decline of normal aging and the more serious decline of dementia. Accordingly, the invention provides a method of preventing, reducing or reversing cognitive impairment (e.g. mild cognitive impairment) in a subject diagnosed as having, or at risk of developing, a proteinopathy. The method comprises administering to the subject an effective amount of a quinuclidine compound as described herein. In related aspects, the invention provides a quinuclidine compound as described herein for use in a method of preventing, reducing or reversing cognitive impairment (e.g. mild cognitive impairment) in a subject diagnosed as having, or at risk of developing, a proteinopathy. In other related aspects, the invention provides the use of a quinuclidine compound as described herein in the manufacture of a medicament for use in a method of preventing, reducing or reversing cognitive impairment (e.g. mild cognitive impairment) in a subject diagnosed as having, or at risk of developing, a proteinopathy.

The methods of the invention can prevent, reduce or reverse loss of cognitive function, autonomic function and/or motor function. In one embodiment, the loss of neural function comprises loss of cognitive function. In certain embodiments, the method prevents, reduces or reverses deterioration in cognitive domains in a subject, e.g. the method prevents, reduces or reverses deterioration in attention and concentration, executive functions, memory (e.g. working memory), language, visuo-constructional skills, conceptual thinking, calculations, orientation, decision making, problem solving, and the like. In one embodiment, the loss of neural function comprises loss of autonomic
function. In certain embodiments, the method prevents, reduces or reverses orthostatic hypotension, constipation, dysphagia, nausea, hypersalivation, hyperhidrosis, urinary dysfunction, sexual dysfunction, and the like. In one embodiment, the loss of neural function comprises loss of motor function. In certain embodiments, the method prevents, reduces or reverses Parkinsonism. Parkinsonism is a clinical definition of a variety of underlying pathologies that can result in Parkinson’s-like symptoms; these pathologies are caused by a number of disorders, including Parkinson’s disease. Symptoms of Parkinsonism which may be prevented, reduced or reversed by the methods disclosed herein include, for example, motor dysfunctions such as tremor, bradykinesia, rigidity, dysphagia, hyperhidrosis, increased sexual dysfunction, and the like.

In one embodiment, the subject does not have protein aggregates comprising α-synuclein in their central nervous system, e.g., in neurons of the substantia nigra, cerebral cortex, ax, hippocampus, frontal lobes and/or temporal lobes. In one embodiment, the proteinopathy is Parkinson’s disease, characterised by the presence of protein α-tau, but not α-synuclein, in within protein aggregates in the central nervous system of the subject, e.g. in neurons of of the substantia nigra, cerebral cortex, hippocampus, frontal lobes and/or temporal lobes of the subject.

The methods of the invention may be beneficial for subjects who have been diagnosed with a proteinopathy, but are not yet experiencing the typical symptoms associated with the disease state, e.g., signs of cognitive impairment. Methods of the invention may also be beneficial for subjects who are at risk of developing a proteinopathy due to, for example, a mutation in the subject or the subject’s family lineage known to cause a proteinopathy. In one embodiment of the methods described herein, the subject has been diagnosed as being at risk of developing said proteinopathy, and the method prevents or delays the onset and/or development of the proteinopathy in the subject.

For example, mutations in the glucocerebrosidase (GAL) gene (GBA), which can cause α-lysosomal storage disease (Gaucher), are known to be associated with an increased risk of developing certain proteinopathies. Mutations in GBA are known in the art to include, for example, L444P, D409H, D409V, E235A, and E340A. Accordingly, in one embodiment the subject to be treated by a method of the invention has one or more of these mutations in GBA. In one embodiment, the subject suffers from a lysosomal storage disease.
A disease such as, for example, Gaucher, Fabry, G\textsubscript{M}\textsubscript{1} gangliosidosis, G\textsubscript{M}\textsubscript{2} Activator deficiency, Tay-Sachs or Sandhoff. In one embodiment, the subject suffers from Gaucher. In an alternative embodiment, the subject to be treated by a method of the invention does not suffer from a lysosomal storage disease such as, for example, Gaucher, Fabry, G\textsubscript{M}\textsubscript{1} - gangliosidosis, G\textsubscript{M}\textsubscript{2} Activator deficiency, Tay-Sachs or Sandhoff. In one embodiment, the subject does not suffer from Gaucher. In a related embodiment, the subject has one (or more than one) mutation in GBA1 but does not suffer from a lysosomal storage disease (e.g. Gaucher). For example, the subject may be a heterozygous carrier for an GBA1 mutation. In another embodiment, the subject does not have a deleterious GBA1 mutation, or, e.g. the gene functions substantially normally in that it encodes a protein with essentially the same structure, activity and/or tissue levels and distribution as the protein encoded by the wild-type gene. Wild-type GBA1 sequences are known in the art and include the GenBank accession number NM_000157.3 (mRNA). In one embodiment, the subject does not have a D409V mutation in GBA1.

Pharmaceutical compositions

The present disclosure also provides pharmaceutical compositions comprising at least one quinuclidine compound as described herein and at least one pharmaceutically acceptable excipient, e.g. for use, according to the methods disclosed herein. The pharmaceutically acceptable excipient can be any such excipient known in the art including those described in, for example, Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro, editor, 1985). Pharmaceutical compositions of the compounds presently disclosed may be prepared by conventional means known in the art, including, for example, mixing at least one presently disclosed compound, with a pharmaceutically acceptable excipient, e.g.

Thus, in one aspect, the invention provides a pharmaceutical dosage form comprising a quinuclidine compound as described herein and a pharmaceutically acceptable excipient, e.g. wherein the dosage form is formulated to provide, when administered (e.g. when administered orally), an amount of said compound sufficient to prevent, reduce or reverse the accumulation of protein aggregates in tissue of a subject, (e.g. a human subject) having or being at risk of developing a proteinopathy. The tissue of the subject may be a neuron of the substantia nigra, cerebral cortex, hippocampus, frontal, temporal lobes, and/or temporal lobes.
In one embodiment, the dosage form is formulated to provide an amount of said quinuclidine compound sufficient to prevent, reduce or reverse the accumulation of protein tau-containing aggregates in tissue of a subject diagnosed as having, or being at risk of developing, Parkinson’s disease. In another embodiment, the dosage form is formulated to provide an amount of said quinuclidine compound sufficient to prevent, reduce or reverse the accumulation of α-synuclein-containing aggregates in tissue of a subject diagnosed as having, or being at risk of developing, Parkinson’s disease, Lewy Body Dementia or Alzheimer’s disease, e.g., Lewy Body Dementia.

A pharmaceutical composition or dosage form of the invention can include an agent and/or another carrier, e.g., compound or composition, inert or active, such as a detectable agent, int, label, adjuvant, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, ve, adjuvant or the like. Carriers also include pharmaceutical excipients and additives, for example, proteins, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, and oligosaccharides; derivatized sugars such as alditols; α, β, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), as, which can be present singly or in combination, comprising alone or in combination, up to 99.99%, by weight or volume. Exemplary proteins include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acid/antibody components, which can also function in an a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, id, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, the, aspartame, and the like. Carbohydrate excipients are also intended within the scope of this invention, examples of which include but are not limited to monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, es, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrose, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol-sorbitol, (glucitol), and myo-inositol, 0 l.

Carriers, which may be used include a buffer or a pH adjusting agent. Typically, the buffer is a salt, prepared from an organic acid or base. Representative buffers include organic acid salts, such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or
phosphate buffers. Additional carriers include polymeric excipients/additives such as polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrates (e.g. cyclodextrins, such as 2-hydroxypropyl-β-cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (e.g. polysorbates such as "TWEEN" 20" and "TWEEN" 80"), lipids (e.g. phospholipids, fatty acids), steroids (e.g. cholesterol), and anticholinergics (e.g. orphenadrine, procyclidine, and apomorphine), MAO-B inhibitors (e.g. rasagiline and selegiline), anticholinergics (e.g. orphenadrine, procyclidine). The present disclosure also provides pharmaceutical compositions, and kits comprising said compositions, which contain at least one quinuclidine compound as described herein and at least one further pharmaceutically-active agent. These pharmaceutical compositions and kits may be adapted to allow simultaneous, subsequent and/or separate administration of the quinuclidine compound and the further active agent. For example, the quinuclidine compound and the further active agent may be formulated in separate dosage forms, e.g. in separate tablets, capsules, lyophilisates or liquids, or they may be formulated in the same dosage form, e.g. in the same tablet, capsule, lyophilisate, or liquid. Where the quinuclidine compound and the further active agent are formulated in the same dosage form, the quinuclidine compound and the further active agent may be present substantially in admixture, e.g., within the core of a tablet, or they may be present substantially in discreet regions of the dosage form, e.g., in separate layers of the same tablet. In one embodiment, the pharmaceutical dosage form comprises a further agent and which is capable of treating or preventing a proteinopathy, e.g., a proteinopathy as described herein.

In a further aspect, the present invention provides a pharmaceutical composition comprising: (i) a quinuclidine compound as described herein; (ii) a further active agent; and (iii) a pharmaceutically acceptable excipient. In one embodiment, the further active agent is an agent which is capable of treating or preventing a proteinopathy, e.g., a proteinopathy as described herein. In one embodiment, the further active agent is capable of treating or preventing a proteinopathy when administered orally to a subject.

Examples of further agents capable of treating proteinopathies such as Parkinson's disease include, for example, dopamine precursors (e.g. L-DOPA), dopamine agonists (e.g. bromocriptine, cabergoline, pergolide, pramipexole and apomorphine), MAO-B inhibitors (e.g. rasagiline and selegiline), anticholinergics (e.g. orphenadrine, procyclidine, and apomorphine).
and trihexyphenidyl), enhancers of α-glucocerebrosidase activity (e.g., ambroxol and afegostat) and lamantadine. Examples of agents capable of treating Alzheimer’s include, for example, acetylcholinesterase inhibitors such as tacrine, rivastigmine, galantamine, ne donepezil, and memantine.

In one embodiment, the further active agent is a chaperone. In another embodiment, the chaperone is capable of: (i) restoring or enhancing at least partial wild-type function and/or activity of the protein (which is aberrant in the proteinopathy); (ii) enhancing the formation of a stable molecular conformation of the protein; (iii) inducing trafficking of the protein from the ER to another cellular location, e.g., a native cellular location, thereby preventing ER-β-R-associated degradation of the protein; and/or preventing aggregation of misfolded protein. In a related embodiment, the chaperone restores or enhances at least partial wild-type function and/or activity of the protein. In other embodiments, the chaperone increases the residual activity of a cell (e.g., a cell from a mammal suffering from a proteinopathy, e.g., synucleinopathy, tauopathy, or the like).

The further active agent may, for example, contain a detectable moiety, a detectable marker moiety, a detectable label moiety, and/or a detection moiety. For example, detectable marker moieties include chemical, physical, biological, and/or other means. Exemplary moieties include, but are not limited to, enzymes, fluorescent molecules, particle labels, electron-dense reagents, radiolabels, biotin, digoxigenin, or a hapten or a protein that has been made detectable. The further active agent may, for example, contain an additional chemical and/or biological moiety not normally part of the agent. Those derivatized-ed moieties can improve delivery, solubility, biological half-life, absorption of the agent, and/or the like. The moieties can also reduce or eliminate any undesirable side effects of the agent and the like. An overview for those moieties can be found in Remington’s Pharmaceutical Sciences (20th ed., Mack Publishing Co., 2000) (see also Pathan et al. (2009) Recent Patents on Drug Delivery & Formulation 3:71-89). The agent can be covalently linked to the moiety. In related embodiments, the agent is covalently linked to the moiety. In related embodiments, the covalent linkage of the moiety is N-terminal to a polynucleotide/polypeptide. In related embodiments, the covalent linkage of the moiety is C-terminal to a polynucleotide/polypeptide.
Further therapies for proteinopathies which may be combined with the methods described herein include psychosocial interventions, behavioural interventions, reminiscence therapy, validation therapy, supportive psychotherapy, sensory integration, cognitive retraining, rehabilitation, speech therapy, and the like. Other interventions include surgery, rehabilitation, and diet management.

The presently disclosed quinuclidine compounds and pharmaceutical compositions can be used in an animal or human. Thus, a presently disclosed compound can be formulated as a pharmaceutical composition for oral, buccal, parenteral (e.g. intravenous, intramuscular or subcutaneous), topical, rectal or intranasal administration or in a form suitable for administration by inhalation or insufflation. In particular embodiments, the quinuclidine compound or pharmaceutical composition is formulated for systemic administration, e.g. via a non-parenteral route. In one embodiment, the quinuclidine compound or pharmaceutical composition is formulated for oral administration, e.g. in solid form. Such modes of administration and the methods for preparing appropriate pharmaceutical compositions are described, for example, in Gibaldi's Drug Delivery Systems in Pharmaceutical Care (1st ed., American Society of Health-System Pharmacists 2007).

The pharmaceutical compositions can be formulated so as to provide slow, extended, or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. The pharmaceutical compositions can also optionally contain opacifying agents and may be of a composition that releases the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner, e.g. by using an enteric coating. Examples of embedding compositions include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more pharmaceutically acceptable carriers, excipients, or diluents well known in the art (see, e.g., Remington's). The compounds presently disclosed may be formulated for sustained delivery according to methods well known to those of ordinary skill in the art. Examples of such formulations can be found in United States Patents 3,119,742; 3,492,397; 3,538,214; 4,060,598; and 4,173,626.
In solid dosage forms for oral administration (e.g. capsules, tablets, pills, dragees, es, powders, granules and the like), the active ingredient(s) are mixed with one or more pharmaceutically acceptable carriers, excipients, or diluents, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, microcrystalline cellulose, calcium phosphate and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, se, alginates, gelatin, pregelatinized maize starch, polyvinylpyrrolidone, hydroxypropyl methylcellulose, sucrose and/or sorbitol; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, sodium starch glycolate, etc., potato or tapioca starch, alginic acid, kertatin silicates, and sodium carbonate; (5) solution or retarding agents, such as paraffin; (6) absorption or accelerating agents, such as ammonium compounds; (7) wetting agents, such as, for example, sodium lauryl sulphate, etc., acetyl alcohol and glycol-sorbitan monoesters; (8) absorbents, such as kaolin and bentonite; etc., clay; (9) lubricants, such as talc, silica, calcium stearate, magnesium stearate, solid lid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets, and pills, the pharmaceutical compositions can also comprise buffering agents. Solid compositions of a similar type can also be prepared using fillers, in soft and hard-filled gelatin capsules, and excipients such as lactose or milk sugar, as well as high molecular weight polyethylene glycols and the like, etc.

A tablet can be made by compression or molding, optionally with one or more accessory or auxiliary ingredients. Compressed tablets can be prepared using binders, such as, for example, gelatin, or hydroxypropylmethylcellulose, lubricants, inert diluents, preservatives, disintegrants, etc., (for example, sodium starch glycolate or cross-linked sodium carboxymethylcellulose), etc., surface-actives, and/or dispersing agents. Molded tablets can be made by molding in a suitable machine, a mixture of the powdered active ingredient, moistened with an inert or liquid diluent. The tablets and other solid dosage forms, such as dragees, capsules, pills, and granules, can optionally be scored or prepared with coatings and shells, such as, as enteric coatings, and other coatings, well known in the art.

In embodiments, the pharmaceutical compositions are administered orally in a liquid aid form. Liquid dosage forms for oral administration of an active ingredient include pharmaceutical acceptable emulsions, microemulsions, solutions, suspensions, syrups, etc.
and elixirs. Liquid preparations for oral administration may be presented as a dry product for constitution with water or other suitable vehicle before use. In addition to the active ingredient, the liquid dosage forms can contain inert diluents commonly used in the art, e.g., such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (e.g., cottonseed, groundnut, corn, linseed, germ, olive, castor and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. In addition to inert diluents, the liquid pharmaceutical compositions can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents, and the like. Suspensions, in addition to the active ingredient(s), can contain suspending agents such as, but not limited to, ethoxylated soya bean oil, polysorbate 80 (polyoxyethylene sorbitan monooleate), microcrystalline cellulose, aluminum metaphosphate, bentonite, agar-agar and tragacanth, and mixtures thereof. Suitable liquid preparations may be prepared by conventional means with one pharmaceutically acceptable additive(s) such as: suspending agent (e.g., sorbitol syrups, methyl cellulose or hydrogenated edible fats); emulsifying agent (e.g., deciniolein acacia); non-aqueous vehicle cle (e.g., almond oil, oily esters or ethyl alcohol); and or preservative (e.g., methyl or propyl parabens - p-hydroxybenzoates or sorbic acid). The active ingredient(s) can also be administered as a bolus, electuary, or paste.

For buccal administration, the composition may take the form of tablets or lozenges formulated in a conventional manner.

In embodiments, the pharmaceutical compositions are administered by non-oral means such as, by topical application, transdermal application, injection, and the like. In related embodiments, the pharmaceutical compositions are administered parenterally by injection, infusion, or implantation (e.g., intravenous, intramuscular, intraperitoneal, and subcutaneous, and the like).

Presently disclosed compounds may be formulated for parenteral administration by injection, including using conventional catheterization techniques, or infusion. Formulations for injection may be presented in unit dosage forms, e.g., in ampules or in multi-dose containers, with an added preservative. The compositions may take such forms as boluses, electuary, or paste.
as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain in a formulating agent such as a suspending, stabilizing and/or dispersing agent recognized by those of skill in the art. Alternatively, the active ingredient may be in powder form for reconstitution with a suitable vehicle, e.g. sterile pyrogen-free water, before use.

The pharmaceutical compositions may be administered directly to the central nervous system. Accordingly, in certain embodiments the composition is administered directly to the central nervous system so as to avoid the blood brain barrier. In some embodiments, the composition can be administered via direct spinal cord injection. In some embodiments, the composition is administered by intrathecal injection. In some embodiments, the composition is administered via intracerebroventricular injection. In embodiments, the composition is administered into a cerebral lateral ventricle. In embodiments, the composition is administered into both cerebral lateral ventricles. In additional embodiments, the composition is administered via intrahippocampal injection. The compositions may be administered in one injection or in multiple injections. In other embodiments, the composition is administered to one more than one location (e.g. to two sites in the central nervous system).

The pharmaceutical compositions can be in the form of sterile injections. The pharmaceutical compositions can be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. To prepare such a composition, the active ingredient is dissolved or suspended in a parenterally acceptable liquid vehicle. Exemplary vehicles include, but are not limited to, water, water adjusted to a suitable pH by addition of an appropriate amount of hydrochloric acid, sodium hydroxide or a suitable buffer, 1,3-butanediol, Ringer's solution and isotonic sodium chloride solution. The pharmaceutical composition can also contain one or more preservatives, for example, methyl, ethyl, n-propyl p-hydroxybenzoate. To improve solubility, a dissolution enhancer or solubilizing agent can be added or the solvent can contain 10-60% w/w of propylene glycol or the like.

The pharmaceutical compositions can contain one or more pharmaceutically acceptable solutions, suspensions, emulsions, as well as sterile, isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions.
or sterile powders, which can be reconstituted into sterile injectable solutions or dispersions just prior to use. Such pharmaceutical compositions can contain antioxidants, 
buffer, bacteriostats, solutes, which render the formulation isotonic with the blood of the intended recipient; suspending agents; thickening agents; preservatives; and the like, e.g.

5 Examples of suitable aqueous and nonaqueous carriers, which can be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, e.g., such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. In some embodiments, in order to prolong the effect of an active ingredient, it is desirable to slow the absorption of the compound from a subcutaneous or intramuscular injection. This can be accomplished by the use of a liquid fluid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the active ingredient then depends upon its rate of dissolution which, in turn, can depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered active ingredient is accomplished by dissolving or suspending the compound in a nonoil vehicle. In addition, prolonged absorption of the injectable pharmaceutical form can be brought about by the inclusion of agents that delay absorption, such as aluminum monostearate and gelatin.

Controlled release parenteral compositions can be in form of aqueous suspensions, microspheres, microcapsules, magnetic microspheres, oil solutions, oil suspensions, emulsions, or the active ingredient can be incorporated in a biocompatible carrier(s). Materials for use in the preparation of microspheres and/or microcapsules include, but are not limited to, biodegradable/bioerodible polymers such as polyglactin, poly-(isobutylcyanoacrylate), e.g., poly(2-hydroxyethyl-L-glutamine), and poly(lactic acid). Biocompatible carriers which can be used when formulating a controlled release parenteral formulation include de carbohydrates, such as dextrans, proteins, such as albumin, lipoproteins or antibodies, e.g., Materials for use in implants can be non-biodegradable, e.g., polydimethylsiloxane, or
biodegradable such as, e.g., poly(caprolactone), poly(lactic acid), poly(glycolic acid) or poly(orthoesters).

For topical administration, the presently disclosed compound may be formulated as an ointment or cream. Presently disclosed compounds may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional anal suppository bases such as cocoa butter or other glycerides.

For intranasal administration or administration by inhalation, presently disclosed compositions may be conveniently delivered in the form of a solution or suspension from an a pump spray container that is squeezed or pumped by the patient or as an aerosol spray. A pressurized container or a nebulizer, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dioxane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container or nebulizer may contain a solution or suspension of the presently disclosed compound. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of a presently disclosed compound and a suitable powder base such as lactose or starch.

Generally, the agents and compositions described herein are administered in an effective amount or quantity, sufficient to treat or prevent a proteinopathy in a subject. Typically, the dose can be adjusted within this range, based on, e.g., age, physical condition, body weight, sex, diet, time of administration, and other clinical factors. Determination of an effective amount is well within the capability of those skilled in the art.

A proposed dose of a quinuclidine compound, as described herein, for oral, parenteral, or buccal administration to the average adult human for the treatment or prevention of a proteinopathy is about 0.1 mg to about 2000 mg. In certain embodiments, the proposed dose is from about 0.2 mg to about 1000 mg of the active ingredient per unit dose. Irrespective of the amount of the proposed dose, administration of the compound can occur, for example, 1 to 4 times per day. In one embodiment the dose, for oral or intranasal administration, is about 0.5 to about 2000 mg, e.g., about 1 to about 750 mg. In one embodiment...
embodiment the dose for direct administration into the central nervous system is about 1-10 \text{mg}, e.g. about 0.5-10 \text{mg}.

Aerosol formulations for the treatment or prevention of the conditions referred to above may be arranged so that each metered dose or “puff” of aerosol contains about 1 \text{mg} to about 10 \text{mg}, e.g. about 5 \text{mg} to about 0.5 \text{mg}, or about 10 \text{mg} to about 0.1 \text{mg}.

In other aspects, the invention provides a dosage form or pharmaceutical composition as described herein for use in therapy, e.g. for use in a method as defined herein.

Having been generally described herein, the following non-limiting examples are provided to further illustrate this invention.

**EXAMPLES: S**

**General procedures for chemical synthesis**

**General Procedure A: Carbamate formation with triphosgene**

To a suspension of amine hydrochloride (1 equivalent) and triethylamine (3-4 equivalents) in THF (concentration ~0.2M) at room temperature was added triphosgene (0.35 equivalents). The reaction mixture was stirred for 10 min and a small amount of ether ether (1-2 mL) was added. The triethylammonium salt was filtered off to afford a clear solution of isocyanate in THF/ether.

To a solution of alcohol (1.5 equivalents) in THF (concentration ~0.2M) at room temperature was added NaN\textsubscript{(60\%, oil)} (1.5 equivalents). The reaction mixture was stirred for 15 min and the above solution (isocyanate in THF/ether) was added dropwise. The reaction was quenched with brine. The solution was extracted with EtOAc and the organic layer was dried, filtered and concentrated. The crude material was purified on combiflash (SiO\textsubscript{2} cartridge, CHCl\textsubscript{3} and 2 N NH\textsubscript{3} in MeOH) to afford the corresponding carbamate.

**General Procedure B: Alkylation with organocerium**
A suspension of CeCl₃ (4 equivalents) in THF (concentration ~ 0.2 M) was stirred at room temperature for 1 h. The suspension was cooled to -78 °C and MeLi/Ether (1.6 M) was added dropwise. The organocerium complex was allowed to form for a period of 1 h and a solution of nitrile (1 equivalent) in THF (concentration ~ 0.2 M) was added dropwise. The reaction mixture was warmed up to room temperature and stirred for 18 h. The solution was cooled to 0 °C and quenched with water (~1 mL) followed by addition of 50% aqueous solution of ammonium hydroxide (~3 mL) until precipitated tetracarbonyl titanium was formed and settled to the bottom of the flask. The mixture was filtered through a pad of Celite and concentrated. The crude material was treated with a solution of HCl/dioxane (4.0 M). The intermediate arylprop-2-amine hydrochloride was triturated in ether and used as is for the next step. Alternatively, the crude free base amine was purified on a combiflash (SiO₂, cartridge, CHCl₃ and 2 N NH₃ in MeOH) to afford the corresponding coupling adduct.

General Procedure C: Suzuki coupling

To a solution of aryl halide (1 equivalent) in a mixture of DME/water [4:1] (concentration ~ 0.2 M) was added boronic acid (2 equivalents), palladium catalyst (0.1-0.25 equivalent) and sodium carbonate (2 equivalents). The reaction mixture was microwaved 25 min at 150 °C. After filtering through a Celite plug and concentrating, the crude product was purified on a combiflash (SiO₂, cartridge, CHCl₃ and 2 N NH₃ in MeOH) to afford the corresponding coupling adduct.

Alternatively: To a solution of aryl halide (1 equivalent) in a mixture of toluene/water [20:1] (concentration ~ 0.2 M) was added boronic acid (1.3-2.5 equivalents), palladium catalyst (0.05-0.15 equivalent), tricyclohexylphosphine (0.15-0.45 equivalent) and potassium phosphate (5 equivalents). The reaction mixture was microwaved 25 min at 150 °C. After filtering through a Celite plug and concentrating, the crude product was purified on a combiflash (SiO₂, cartridge, CHCl₃ and 2 N NH₃ in MeOH) to afford the corresponding coupling adduct.

General Procedure D: Cyclopropanation

To a mixture of aryl nitrile (1 equivalent) and Ti(Oi-Pr)₅ (1.7 equivalents) stirring at -70 °C, was added dropwise EtMgBr [3.0 M in ether] (1.1 equivalents). The reaction on
mixture was allowed to warm to 25°C and stirred for 1 h. To the above mixture was added BF₃·Et₂O (3 equivalents) dropwise at 25°C. After the addition, the mixture was stirred for another 2 h, and then quenched with aqueous HCl (2 M). The resulting solution was then basified by adding aqueous NaOH (2 M). The organic material was extracted with ethyl ether. The organic layers were combined, dried over Na₂SO₄, filtered and concentrated. The crude material was purified by silica gel column chromatography by (eluting with petroleum ether/EtOAc::10/1 to 1/1) to give the corresponding 1-aryl-1-cyclopropanamine.

10 General Procedure E: Biaryl coupling using Suzuki conditions

To a stirred solution of the aryl halide component (1 equivalent) in 5:1 (v/v) dioxane/water (~0.15 M), or 5:1 (v/v) N₂N-dimethylformamide (~0.15 M), was added the arylboronate or arylboronic acid component (1-1.5 equivalents), sodium carbonate (2-3-3 equivalents) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (0.05 equivalents). The mixture was heated to 90°C overnight and then filtered through a plug of Celite. The Celite was rinsed with ethyl acetate and the combined filtrate was washed with brine, dried (Na₂SO₄) and concentrated. The residue was purified by flash chromatography on silica gel.

General Procedure F: Carbamate formation using an isocyanate generated via a mixed anhydride/Curtius Rearrangement route

To a stirred solution of the carboxylic acid component (1 equivalent) in tetrahydrofuran (-0.1 M) was added triethylamine (2 equivalents). The reaction was cooled to 0°C and then treated with isobutyl chloroformate (1.5 equivalents). After 1 hour at 0°C, a solution of sodium azide (2 equivalents) in water (~1 M) was added and the reaction was allowed to warm to room temperature. After overnight stirring, the reaction was diluted with water, filtered and extracted with ethyl acetate. The combined extracts were washed with aqueous sodium bicarbonate solution and brine, dried (Na₂SO₄), and concentrated. The crude acyl cyanide azide was further dried via coevaporation with toluene and then taken up in toluene (~0.1 M). The stirred solution was refluxed for 2-2.5 hours, cooled and treated with an alcohol (1:10) component (1.25-2 equivalents). The reaction was heated at reflux overnight and then...
concentrated. The residue was taken up in either ethyl acetate or chloroform and washed with aqueous sodium carbonate, \( \text{Na}_2\text{CO}_3 \), and concentrated. The crude product was purified by flash chromatography over silica using chloroform/methanol (less polar carbamates) or chloroform/methanol/ammonia (more polar carbamates). Solvent gradients.

Example 1: 
1-azabicyclo[2.2.2]oct-3-yl\[2-(4′-fluorobiphenyl-3-yl)propan-2-\]-ylcarbamate ([Compound 1])

Using General Procedure C, 1-azabicyclo[2.2.2]oct-3-yl\[2-(3-bromophenyl)propan-2-\]-ylcarbamate (600 mg, 1.63 mmol), 4-fluoro phenylboronic acid (457 mg, 3.27 mmol) and palladium(II) acetate gave the title compound as a white solid (373 mg; 60%). \( \text{H}^1 \text{NMR} \) (400 MHz, CDCl$_3$) \( \delta \) 7.56 (s, 1H), 7.52 (dd, \( J = 5.4, 8.4 \) Hz, 2H), 7.42-7.38 (m, m, 3H), 7.12 (m, 2H), 5.18 (s, 1H), 4.62 (s, 1H), 2.66 (m, 6H), 1.72 (s, 6H), 2.01-0.83 (m, m, 5H) ppm. \( \text{C NMR} \) (100 MHz, CDCl$_3$) \( \delta \) 125.0, 124.0, 123.8, 116.0, 116.0, 71.3, 55.9, 55.5, 47.6, 46.7, 29.6, 25.6, 24.8 ppm. Purity: 98.0% UPLCMS (210 nm); retention time: 0.95 min; \( \text{M}^+ \text{} = 382.9 \) (analog for C$_{17}$H$_{24}$FN$_2$O$_2$, 0.37 (CHCl$_3$); C, 65.86; H, 6.47; N, 6.57. Found: C, 65.85; H, 6.69; N, 6.49.

Example 2: 
(5)-quinuclidin-3-yl\[2-(4-fluorophenyl)thiazol-4-yl]propan-2-\]-ylcarbamate ([Compound 2])

To a stirred solution of 4-fluoro thiobenzamide (8.94 g, 57.6 mmol) in ethanol (70 mL), a solution of ethyl 4-chloroacetoacetate (7.8 mL, 58 mmol) was added. The reaction was heated at reflux for 4 hours. A 1:1 mixture of ethyl 4-chloroacetoacetate (1.0 mL, 7.4 mmol) was added, and the reaction mixture was stirred for 4 hours. The reaction mixture was then concentrated, and the residue was partitioned between ethyl acetate (200 mL) and aqueous NaHCO$_3$ (200 mL). The organic layer was combined with a back extractions of the aqueous layer (ethyl acetate, 1 x 75 mL), dried (Na$_2$SO$_4$), and concentrated. The resulting amber oil was purified by flash chromatography using hexane/ethyl acetate gradient to afford ethyl 2-(2-(4-fluorophenyl)thiazol-4-yl)acetate, as a low melting, nearly colourless solid (13.58 g, 89%).

To a stirred solution of ethyl 2-(2-(4-fluorophenyl)thiazol-4-yl)acetate (6.28 g, 23.73 mmol) in DMF (50 mL), sodium hydride (60% dispersion in mineral oil) (2.84 g, 71.0 mmol) was added. The frothy mixture was stirred for 15 minutes before cooling in an ice bath.
bath and adding iodomethane (4.4 mL, 71 mmol). The reaction was stirred overnight, allowing the cooling bath to slowly warm to room temperature. The mixture was then concentrated and the residue partitioned between ethyl acetate (80 mL) and water (200 mL). The organic layer was washed with a second portion of water (1 x 200 mL), dried (Na$_2$SO$_4$) and concentrated. The resulting amber oil was purified by flash chromatography using a chloroform/methanol/ammonia gradient to afford the title compound 2-(2-(4-fluorophenyl)thiazol-4-yl)-2-methylpropanoic acid as a colourless oil (4.57 g, 71%, 66%).

To a stirred solution of ethyl 2-(2-(4-fluorophenyl)thiazol-4-yl)-2-methylpropanoate (4.56 g, 15.5 mmol) in 1:1 THF/ethanol/water (45 mL) was added lithium hydroxide monohydrate (2.93 g, 69.8 mmol). The reaction was stirred overnight, concentrated and the residue redissolved in water (175 mL). The solution was washed with ether (1 x 100 mL), acidified by the addition of 1.0 N HCl (80 mL) and extracted with ethyl acetate (2 x 70 mL). The combined extracts were dried (Na$_2$SO$_4$) and concentrated to afford ethyl 2-(2-(4-fluorophenyl)thiazol-4-yl)-2-methylpropanoate as a white solid (4.04 g, 98%, 96%). This material was used in the next step without purification.

To a stirred and cooled (0 °C) solution of 2-(2-(4-fluorophenyl)thiazol-4-yl)-2-methylpropanoic acid (4.02 g, 15.2 mmol) in THF (100 mL) was added trimethylamine (3.0 mL, 23 mmol). The reaction was stirred cold for another 1 hour before adding a solution of sodium azide (1.98 g, 30.5 mmol) in water (20 mL). The reaction was stirred overnight, allowing the cooling bath to slowly warm to room temperature. The mixture was then diluted with water (100 mL) and extracted with ethyl acetate (2 x 60 mL). The combined extracts were washed with 1 M aqueous NaHCO$_3$, 1 M water, and brine (1 x 100 mL), dried (Na$_2$SO$_4$) and concentrated. After coevaporating with toluene (2 x 50 mL), the resulting white solid was taken up in toluene (100 mL) and refluxed for 4 hours. (S)-3-quinuclidinol (3.87 g, 30.4 mmol) was then added and reflux was continued overnight. The reaction was concentrated and the residue partitioned between ethyl acetate (100 mL) and aqueous NaHCO$_3$ (1.50 mL). The organic layer was washed with water (1 x 150 mL), dried (Na$_2$SO$_4$) and concentrated. The resulting off-white solid was purified by flash chromatography using a chloroform/methanol/ammonia gradient, to afford the title compound 2-(2-(4-fluorophenyl)thiazol-4-yl)-2-methylpropanoate as a white solid (4.56 g, 98%, 66%).
compound as a white solid (4.34 g, 73%).

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.96-7.88 (m, 2H), 7.16-7.04 (m, 3H), 5.55 (brs, 1H), 4.69-4.62 (m, 1H), 3.24-3.11 (m, 1H), 3.00-2.50 (m, 5H), 2.01-1.26 (m, 11H) ppm.

$^{13}$C NMR (400 MHz, CDCl$_3$): $\delta$ 166.4, 165.1, 163.8 (d, J = 250.3 Hz), 162.9, 155.0, 130.1 (d, J = 3.3 Hz), 128.4 (d, J = 8.5 Hz), 115.9 (d, J = 22.3 Hz), 112.5, 71.2, 55.7, 54.2, 47.5, 46.5, 28.0, 25.5, 24.7, 19.6 ppm. Purity: 100%.

UPLCMS (210 nm & 254 nm): retention time 0.83 min; (M+1) 390.
Example 3: (S)-quiniclidin-3-yl[2-(4’-methylpropanoic acid)-2-methylpropanoate][2-(4’-methylpropanoic acid)-2-methylpropanoate] carbamate (Compound 3)

5 Using General Procedure E and the reaction inputs ethanol, 2-(4-bromophenyl)-2-methylpropanoate and 4-(2-methylpropanoic acid), ethyl 2-(4’-2-methylpropanoic acid)-2-methylpropanoate was prepared as an off-white solid. To a stirred solution of this compound (3.01 g, 8.78 mmol) in 1:1:1 (v/v/v) toluene/ethanol/water (45 mL) was added lithium hydroxide monohydrate (1.47 g, 61.4 mmol). The mixture was heated at reflux overnight and then concentrated. The residue was dissolved in water, treated with 1 N hydrochloric acid (65 mL) and extracted with ethyl acetate. The combined organic layers were washed with brine, dried (Na₂SO₄) and concentrated to afford 2-(4’-methylpropanoic acid)-2-methylpropanoic acid as a white solid (2.75 g, 100%). This intermediate and nd (S)-quiniclidin-3-ol were reacted according to: General Procedure F to generate the title compound as a colourless, glassy solid. [1H NMR (400 MHz, DMSO-d₆)] δ 7.62-7.29 (m, 7H), 7.01 (d, J = 8.9 Hz, 2H), 4.47-4.37 (m, 1H), 4.17-4.08 (m, 2H), 3.72-3.62 (m, 1H), 3.32 (s, 3H), 3.09-2.25 (m, 6H), 2.05-1.18 (m, 11H) ppm. [13C NMR (100 MHz, DMSO-d₆)] δ 157.9, 154.5, 146.7, 137.4, 132.5, 127.5, 125.7, 125.2, 114.8, 70.4, 70.0, 66.9, 58.2, 55.4, 54.2, 46.9, 45.9, 29.4, 25.3, 24.2, 19.2 ppm. Purity: 100%, 100% (210 & 254 nm) UPLCMS; retention time: 0.87 min; (M+H)⁺ 439.5.

Example 4: 1-azabicyclo[2.2.2]oct-3-yl[2-(biphenyl-3-yl)propan-2-yl]carbamate (Compound 4)

Using General Procedure C, 1-azabicyclo[2.2.2]oct-3-yl[2-(3-bromophenyl)propan-2-yl]carbamate (600 mg, 1.63 mmol), phenylboronic acid (398 mg, 3.27 mmol) and nd palladium(II)acetate gave the title compound as a white solid (379 mg, 64%). [1H NMR (400 MHz, CDCl₃)] δ 7.61 (s, 1H), 7.56 (d, J = 7.4 Hz, 2H), 7.50-7.38 (m, 4H), 7.34 (m, 2H), 5.16 (s, 1H), 4.63 (s, 1H), 3.39-2.09 (m, 6H), 1.72 (s, 6H), 0.20-0.73 (m, 5H) ppm. Purity: 99% UPLCMS (210 nm); retention time: 0.84 min; (M+1) 365.0. Anal. Calcd. for C₃₂H₃₄N₂O₂, 0.29 (CHCl₃); C, 70.02; H, 7.14; N, 7.01. Found: C, 70.02; H, 7.37; N, 6.84.
Example 5: (S)-quinuclidin-3-yl 2-(biphenyl-4-yl)propan-2-ylcarbamate (Compound 5)

Using General Procedure B, bromobenzonitrile (2.00 g, 11.0 mmol) was converted to the corresponding 2-(4-bromophenyl)propan-2-amine (1.20 g, 51%) as a brown oil.

Using General Procedure A, 2-(4-bromophenyl)propan-2-amine (1.0 g, 4.7 mmol) and (S)-quinuclidin-3-ol gave (S)-quinuclidin-3-yl 2-(4-bromophenyl)propan-2-ylcarbamate (1.00 g, 58%) as a brown oil.

Using General Procedure C, the above bromide (200 mg, 0.540 mmol), phenylboronic acid (133 mg, 1.10 mmol) and [PdCl₂(pddf)CH₂Cl₂] gave the title compound as a white solid (70 mg, 35%).

\[ \text{H NMR (500 MHz, CDCl₃):} \]
\[ \delta 7.60-7.53 (m, 4H), 7.47 (d, J = 8.5 Hz, 2H), 7.42 (t, J = 7.5 Hz, 2H), 7.33 (t, J = 7.5 Hz, 1H), 5.26 (br s, 1H), 4.64 (m, 1H), 3.33-3.15 (m, 1H), 3.10-2.45 (m, 5H), 2.40-1.80 (m, 2H), 1.78-1.58 (m, 7H), 1.55-1.33 (m, 2H) \] ppm.

\[ \text{13C NMR (125 MHz, CDCl₃):} \]
\[ \delta 154.5, 146.1, 140.8, 139.5, 128.7, 127.2, 127.1, 127.1, 127.1, 125.2, 70.9, 75.5, 75.5, 147.4, 144.6, 31.1, 29.5, 25.3, 24.5, 19.5 \] ppm.

Purity: 100%

LCMS (214 nm & 254 nm): retention time 1.56 min; (M+1) 365.

Example 6: Quinuclidin-3-yl 1-(biphenyl-4-yl)cyclopropylcarbamate (Compound 6)

Using General Procedure D, bromobenzonitrile (3.00 g, 16.5 mmol) was converted to the corresponding 1-(4-bromophenyl)cyclopropanamine (1.80 g, 51%) as a yellow solid.

Using General Procedure A, 1-(4-bromophenyl)cyclopropanamine (1.0 g, 4.7 mmol) and (S)-quinuclidin-3-ol gave quinuclidin-3-yl 1-(4-bromophenyl)cyclopropyl-carbamate (1.3 g, 75%) as a white semi-solid.

Using General Procedure C, the above carbamate (400 mg, 1.2 mmol), phenylboronic acid (267 mg, 2.22 mmol) and [PdCl₂(pddf)]CH₂Cl₂ gave the title compound as a viscous oil (100 mg, 25%).

\[ \text{H NMR (500 MHz, CDCl₃):} \]
\[ \delta 7.47 (d, J = 7.5 Hz, 2H), 7.43 (d, J = 8.08 Hz, 2H), 7.33 (t, J = 7.5 Hz, 2H), 7.26-7.15 (m, 3H), 5.93 (brs, 0.6H), 5.89 (brs, 0.4H), 4.67 (m, 1H), 3.20-3.06 (m, 1H), 2.88-2.42 (m, 5H), 1.98-1.08 (m, 9H) \] ppm.

\[ \text{13C NMR (125 MHz, CDCl₃):} \]
\[ \delta 155.0, 146.1, 139.7, 138.2, 137.7, 137.7, 137.7, 126.1, 126.0, 124.8, 124.1, 70.0 \] ppm.
Example 7: \((S)\)-quinuclidin-3-yl-1-(4'-fluorobiphenyl-4-yl)cyclopropylcarbamate (Compound 7)

Using General Procedure C, \((S)\)-quinuclidin-3-yl-1-(4-bromophenyl)cyclopropylcarbamate, 4-F-phenylboronic acid and \(\text{PdCl}_2\left(pddf\right)\)\(\text{CH}_2\text{Cl}_2\) gave the title compound as a white solid (45%). \(^1\)H NMR \((500\text{MHz}, \text{DMSO-}d_6)\) \(\delta 8.06-7.83\ (d, 1H), 7.69-7.66\ (m, 2H), 7.59-7.55\ (m, 2H), 7.29-7.22\ (m, 4H), 4.56-4.54\ (m, 1H), 3.13-2.32\ (m, 6H), 1.91-1.19\ (m, 9H) ppm. \(^13\)C NMR \((125\text{MHz}, \text{DMSO-}d_6)\) \(\delta 163.2, 161.2, 156.4, 143.7, 136.9, 128.9, 128.8, 126.8, 112.6, 111.6, 70.7, 75.8, 47.4, 46.4, 34.8, 25.7, 24.6, 19.6, 18.7, 18.6 ppm. Purity: >97% LCMS \((214\text{nm} & 254\text{nm})\); retention time 1.96 min; \((M+1) 381.2\).

Example 8: \((S)\)-1-azabicyclo[2.2.2]oct-3-yl-[1-(2',4'-difluorobiphenyl-4-yl)cyclopropyl]carbamate (Compound 8)

Using General Procedure C, \((S)\)-quinuclidin-3-yl-1-(4-bromophenyl)cyclopropylcarbamate \((0.446\ g, 1.22\ mmol), 2,4\text{-difluorophenyl boronic acid} \((0.386\ g, 2.44\ mmol)\) and \(\text{Pd(OAc)}_2\) \((0.015\ g, 0.067\ mmol)\) gave the title compound as a tan solid \((0.111\ g, 23\%)\). \(^1\)H NMR \((\text{CDCl}_3)\) \(\delta 7.43\ (dd, \ J=8.4, 1.6\ Hz, 2H), 7.40-7.33\ (m, 1H), 7.31\ (d, \ J=7.7\ Hz, 2H), 6.99-6.81\ (m, 2H), 5.48\ (d, \ J=48.0\ Hz, 1H), 4.82-4.65\ (m, 1H), 4.52\ (s, 1H), 4.13\ (s, 1H), 3.97\ (s, 1H), 1.97\ (s, 1H) ppm. \(^13\)C NMR: major rotomer \((\text{CDCl}_3)\) \(\delta 162.2\ (dd, \ J=12.8, 249.1\ Hz), 159.8\ (dd, \ J=11.8, 251.0\ Hz), 156.9, 156.0, 142.6, 133.1, 131.3\ (m), 128.9, 125.6, 124.9, 111.5\ (dd, \ J=3.9, 21.2\ Hz), 104.4\ (dd, \ J=25.2, 29.4\ Hz), 72.1, 71.6, 65.7, 47.4, 46.5, 35.7, 35.3, 25.5, 24.6, 24.4, 19.5, 18.1 ppm. Purity: LCMS > 99.3% \((214\text{nm} & 254\text{nm})\); retention time 0.90 min; \((M+1) 399.0\).
Example 9: 1-azabicyclo[2.2.2]oct-3-yl[1-(4'-methoxybiphenyl)-4-yl]cyclopropylcarbamate (Compound 9)

Using General Procedure C, quinuclidin-3-yl-1-(4-bromophenyl)cyclopropylcarbamate (0.485 g, 1.33 mmol), 4-methoxyphenyl boronic acid (0.404 g, 2.66 mmol) and Pd(OAc)$_2$ (0.016 g, 0.071 mmol) gave the title compound as a gray solid (0.337 mg, 65%). $^1$H NMR 4R (CDCl$_3$) $\delta$ 7.48 (dd, $J$ = 8.6, 5.5 Hz, 4H), 7.29 (d, $J$ = 7.6 Hz, 2H), 6.96 (d, $J$ = 8.8 Hz, 1H), 2.97-2.42 (m, 5H), 1.97 (d, $J$ = 30.9 Hz, 1H), 1.81 (s, 1H), 1.75-1.33 (m, 3H), 7.29 ppm. i$^1$H NMR [major rotomer (CDCl$_3$)] $\delta$ 159.1, 156.0, 141.4, 4, 139.0, 133.4, 128.0, 126.7, 125.9, 114.2, 71.5, 55.7, 55.3, 47.4, 46.5, 35.3, 25.5, 24.6, 6.6, 19.6, 17.8 ppm. Purity: LCMS > 97.1% (m/z 214 [m/z 254]). Retention time: 0.86 min.; in: (M+1) 393.4.

Example 10: Quinuclidin-3-yl-2-(5-(4-fluorophenyl)thiophen-3-yl)propan-2-ylcarbamate (Compound 10)

To a stirred and cooled (0°C) solution of ethyl 5-bromothiophene-3-carboxylate (13.30 g, 56.57 mmol) in THF (100 mL) was added a solution of methylmagnesium bromide in diethyl ether [3.0 M] (55.0 mL, 165 mmol), dropwise over 20 minutes. After 2 hours, the reaction solution was concentrated. The residue was taken up in aqueous NH$_4$Cl (200 mL) and extracted with ethyl acetate (2 x 100 mL). The combined extracts were dried (Na$_2$SO$_4$) and concentrated. The resulting amber oil was purified by flash chromatography using pentane/ethyl acetate gradient to afford 2-(5-bromothiophen-3-yl)propan-2-ol as a pale amber oil (8.05 g, 64%).

To a stirred solution of 2-(5-bromothiophen-3-yl)propan-2-ol (8.03 g, 36.3 mmol) in methylene chloride (80 mL) was added sodium azide (7.08 g, 109 mmol) followed by trifluoroacetic acid (8.0 mL; dropwise over 5-6 minutes). The thickening suspension was stirred for 1.5 hours before diluting with water (350 mL) and extracting with ethyl acetate (1 x 200 mL). The organic layer was washed with aqueous NaHCO$_3$ (1 x 250 mL), dried (Na$_2$SO$_4$) and concentrated to afford the crude azide product. To a stirred solution of this His material in THF (160 mL) was added water (11 mL) followed by triphenylphosphine in the (23.8 g, 90.7 mmol). The reaction was stirred for 2 days before concentrating. The he
resulting residue was dissolved in ethyl acetate (250 mL) and extracted with 1 N aqueous HCl (4 \times 75 mL). The combined extracts were basified with concentrated NH₄OH and extracted with ethyl acetate (2 \times 100 mL). These extracts were, in turn, dried (Na₂SO₄, 2) and concentrated. The resulting amber oil was purified by flash chromatography using a mixture of ethyl chloroformate/methanol/ammonia gradient to afford a mixture of 2-(5-bromothiophen-3-yl)propan-2-amine and triphenylphosphine oxide (∼70/30 ratio) as a viscous amber oil (1.32 g, 17%).

To a stirred solution of 3-quinuclidinol (3.00 g, 23.6 mmol) in THF (100 mL) was added 4-nitrophenyl chloroformate (5.94 g, 29.5). After stirring for 4 hours, the precipitate was filtered off, rinsed with THF, and air-dried on the frit under house vacuum. The filter cake was dissolved in dimethyl acetate (150 mL) and washed with aqueous NaHCO₃ (1 \times 150 mL) and water (2 \times 150 mL). The organic layer was dried (Na₂SO₄) and concentrated (to afford) a mixture of 4-nitrophenyl 3-yl carbonate, which was used in the next step without purification.

To a stirred solution of 2-(5-bromothiophen-3-yl)propan-2-amine (0.366 g, 1.66 mmol) in THF (10 mL) was added 4-nitrophenyl 3-yl carbonate (0.571 g, 1.95 mmol), and a few granules of 4-(dimethylamino)pyridine. The mixture was refluxed overnight, hot, concentrated, and partitioned between ethyl acetate (50 mL) and aqueous NaHCO₃ (15 mL). The organic layer was washed again with aqueous NaHCO₃ (1 \times 50 mL), and dried (Na₂SO₄) and concentrated. The resulting, dirty yellow gum was purified by flash chromatography using a chloroform/methanol/ammonia gradient to afford quinuclidin-3-3-yl ((1-5-bromothiophen-3-yl) cyclopentyl) carbamate, an off-white solid (0.305 g, 49%).

Using General Procedure C, quinuclidin-3-yl (1-(5-bromothiophen-3-3-yl)cyclopentyl) carbamate (0.227 g, 0.742 mmol), 4-fluorophenylboronic acid (0.208 g, 2.08 mmol), tricyclohexylphosphine (0.021 g, 0.075 mmol), potassium carbonate (0.866 g, 6.08 mmol) and palladium acetate (8.0 mg, 0.00036 mol), gave the title compound as a grey (0.142 g, 49%).

¹H NMR (400 MHz, CDCl₃): δ 7.60-7.45 (m, 2H), 7.24-7.19 (m, 2H), 7.10-6.97 (m, 3H), 5.23 (br s, 1H), 4.72-4.61 (m, 1H), 3.30-3.04 (m, 1H), 3.03-2.25 (m, 5H), 2.09-1.02 (m, 11H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 162.3 (d, δ=247.1 Hz), 154.5, 149.8, 143.6, 130.7, 127.4 (d, δ=8.1 Hz), 121.8, 118.9, 115.8 (d, δ=21.6 Hz).
Hz), 70.8, 55.5, 53.4, 47.3, 46.4, 129.0, 25.4, 24.4, 19.4 ppm. Purity: 95.8% UPLCMS (210 nm & 254 nm); retention time 0.90 min; (M+1) 389.9.

Example 11: (S)-quinuclidin-3-yl)-2-(3-(4-fluorophenyl)isothiazol-5-yl)propan-2-ylcarbamate (Compound 11)

To a stirred solution of 2-(3-(4-fluorophenyl)isothiazol-5-yl)propan-2-amine (1.21 g, 5.12 mmol) in toluene was added a solution of phosgene in toluene of ~1.9 M (10.8 mL, 20.50 mmol). The reaction was heated at reflux for two hours and then concentrated. The residue was coevaporated with toluene (2 x 15 mL) to afford the crude isocyanate intermediate as a golden oil. This material was taken up in toluene (10 mL) and treated with ith

(S)-3-quinuclidinol (0.749 g, 5.89 mmol). The reaction was heated at reflux overnight and then concentrated. The residue was purified by flash chromatography using a chlooroform/methanol/ammonia gradient to afford the title compound as a white solid lid (0.971 g, 49%). \[^\text{1}^\text{H}NMR\ (400\text{MHz},\text{DMSO}-d_6)\] 8.09-8.00 (m, 2H), 7.87 (br, 1H), 7.75 (s, 1H), 7.35-7.25 (m, 2H), 4.54-4.45 (m, 3H), 3.14-2.92 (m, 1H), 2.87-2.17 (m, 5H), 1H, 1.98-0.98 (m, 1H) ppm. \[^\text{13}^\text{C}NMR\ (400\text{MHz},\text{DMSO}-d_6)\] 180.1, 165.6, 162.6 (d, J = 246.4 Hz), 154.7, 131.2 (d, J = 3.0 Hz), 128.7 (d, J = 8.4 Hz), 118.2, 115.7 (d, J = 21.81 Hz), 70.6, 55.3, 52.8, 46.9, 45.9, 29.9, 25.2, 24.2, 19.2 ppm. Purity: 100% UPLCMS (210 nm & 254 nm); retention time 0.82 min; (M+1) 390.0.

Example 12: (S)-quinuclidin-3-yl)-2-(4-(4-fluorophenyl)thiazol-2-yl)propan-2-ylcarbamate (Compound 12)

To a stirred solution of ethyl 3-amino-3-thioxopropanoate (20.00 g, 135.9 mmol) in ethanol (120 mL) was added 2-bromo-4'-fluoracetophenone (29.49 g, 135.9 mmol). The he mixture was refluxed for 1 hour, concentrated, and partitioned between ethyl acetate (300 mL) and aqueous NaHCO\_3 (400 mL). The organic layer was combined with a back extract of the aqueous layer (ethyl acetate, 1 x 100 mL), dried (Na\_2SO\_4) and concentrated. The resulting light brown solid was purified by flash chromatography using a hexane/ethyl acetate gradient to afford ethyl 2-(4-(4-fluorophenyl)thiazol-2-yl)acetate as an off-white solid (29.92 g, 83%).

To a stirred and cooled (-78°C) solution of ethyl 2-(4-(4-fluorophenyl)thiazol-2-2-

yl)acetate (10.00 g, 37.69 mmol) in THF (250 mL) was added a solution of potassium am
t-butoxide in THF [1.0 M] (136 mL, 136 mmol), dropwise over 15 minutes, followed by 18-crown-6 (1.6 mL, 7.5 mmol). After an additional 30 minutes at -78°C, iodomethane (8.5 mL) was added, dropwise over 5 minutes. The reaction was stirred cold for another 2 hours before pouring into water (450 mL) and extracting with ethyl acetate (2 × 150 mL, L). The combined extracts were washed with brine (1 × 200 mL), dried (Na₂SO₄) and concentrated. The resulting brown oil was purified by flash chromatography using a hexane/ethyl acetate gradient to afford ethyl 2-(4-(4-fluorophenyl)thiazol-2-yl)-2-methylpropanoate as a pale amber oil (8.64 g, 78%).

To a stirred solution of ethyl 2-(4-(4-fluorophenyl)thiazol-2-yl)-2-methylpropanoate (0.900 g, 3.07 mmol) in 1:1:1 THF/ethanol/water (15 mL) was added lithium hydroxide monohydrate (0.451 g, 10.7 mmol). After overnight stirring, the reaction was as concentrated and redissolved in water (80 mL). The solution was washed with ether (1 × 150 mL), acidified with the addition of 1 N HCl (15 mL) and extracted with ethyl acetate (2 × 150 mL). The combined extracts were dried (Na₂SO₄) and concentrated to afford 2-(4-(4-fluorophenyl)thiazol-2-yl)-2-methylpropanoic acid as a pale golden solid (0.808 g, 99%).

To a stirred and cooled (0°C) solution of 2-(4-(4-fluorophenyl)thiazol-2-yl)-2-methylpropanoic acid (0.784 g, 2.96 mmol) in THF (25 mL) was added triethylamine (0.82 mL, 5.9 mmol) followed by isobutyl chloroformate (0.58 mL, 4.4 mmol). The reaction was stirred cold for another 1 hour before adding a solution of sodium azide (0.385 g, 5.92 mmol) in water (7 mL). The reaction was stirred overnight, allowing the temperature to slowly warm to room temperature. The mixture was then diluted with water (100 mL) and extracted with ethyl acetate (2 × 60 mL). The combined extracts were washed with aqueous NaHCO₃ (1 × 150 mL) and brine (1 × 100 mL), dried (Na₂SO₄) and concentrated. After coevaporation with toluene (2 × 30 mL), the resulting off-white solid was taken up in toluene (25 mL) and refluxed for 4 hours. (S)-3-Quinuclidinol (0.753 g, 5.92 mmol) was then added, and the reflux was continued for 3 hours. The reaction was as concentrated and the residue was purified by flash chromatography using a chlooroform/methanol/ammonia gradient to afford the title compound as a white solid (0.793 g, 69%). ¹H NMR (400 MHz, CDCl₃) δ 7.90-7.81 (m, 2H), 7.32 (s, 1H), 7.14-7.05 (m, 2H), 5.76 (br s, 1H), 4.72-4.65 (m, 1H), 3.26-3.10 (m, 1H), 2.03-2.37 (m, 1H), 2.05-2.15 (m, 1H), 1.23 (m, 1H) ppm. ¹³C NMR (400 MHz, CDCl₃) δ 177.6, 162.6, 154.8, 158, 178.
153.6, 130.8 (d, \( J = 3.2 \) Hz), 128.1 (d, \( J = 8.1 \) Hz), 115.9 (d, \( J = 21.7 \) Hz), 112.2, 71.6, 55.7, 47.4, 46.5, 29.1, 25.4, 24.7, 19.6 ppm. Purity: 100% UPLCMS (210 nm & 254 nm); retention time: 0.82 min; (M+1) 390.}

Example 13: Quinuclidin-3-yl (2-(4’-(2-methoxyethoxy)-1,1’-biphenyl)-4-yl)propan-2-yl)carbamate (Compound 13)

Using General Procedure F and the reaction inputs 2-(4’-(2-methoxyethoxy)-1,1’-biphenyl)-4-yl)-2-methylpropanoic acid (prepared as described in Example 3) and quinuclidin-3-ol, the title compound was generated as a colourless, glassy solid (23%). NMR data matched that of Example 3. Purity: 100%, 99.1% (210 & 254 nm) UPLCMS; retention time: 0.87 min; (M+H+) 439.0.

Example 14: (S)-quinuclidin-3-yl (2-(3’-(2-methoxyethoxy)-1,1’-biphenyl)-4-yl)propan-2-yl)carbamate (Compound 14)

Exchanging 4-(2-methoxyethoxy)phenylboronic acid for 3-(2-methoxyethoxy)phenylboronic acid, the reaction sequence outlined in Example 3 was used to prepare 2-(3’-(2-methoxyethoxy)-1,1’-biphenyl)-4-yl)-2-methylpropanoic acid. This intermediate and quinuclidin-3-ol were reacted according to General Procedure F to generate the title compound as a glassy, colourless solid. 1H NMR (400 MHz, DMSO-d6) \( \delta \) 7.63-7.31 (m, 6H), 7.24-7.10 (m, 2H), 6.92 (dd, \( J = 8.2, 1.9 \) Hz, 1H), 4.51-4.34 (m, 1H), 4.21-4.08 (m, 2H), 3.72-3.64 (m, 2H), 3.32 (s, 3H), 3.09-2.26 (m, 5H), 2.04-1.22 (m, 9H) ppm. 13C NMR (100 MHz, DMSO-d6) \( \delta \) 158.9, 135.6, 147.6, 141.5, 137.6, 129.9, 126.3, 125.2, 118.9, 113.2, 112.5, 70.4, 70.0, 66.9, 58.2, 55.4, 54.2, 46.9, 45.9, 29.4, 25.3, 24.2 ppm. Purity: 100%, 100% (210 & 254 nm) UPLCMS; retention time: 0.91 min; (M+H+) 439.4.

Example 15: Quinuclidin-3-yl (2-(4’-(2-methoxyethoxy)-1,1’-biphenyl)-3-yl)propan-2-yl)carbamate (Compound 15)

Exchanging ethyl 2-(4-bromophenyl)-2-methylpropanoate for ethyl 2-(3-bromophenyl)-2-methylpropanoate, the reaction sequence outlined in Example 3 was used to prepare 2-(4’-(2-methoxyethoxy)-1,1’-biphenyl)-3-yl)-2-methylpropanoic acid. This intermediate and quinuclidin-3-ol were reacted according to General Procedure F, to generate the title compound.
compound as a yellow solid.  

H NMR (400 MHz, DMSO-d$_6$) $\delta$ 7.62-7.20 (m, 7H), 7.03 (d, $J$ = 8.7 Hz, 2H), 4.48-4.35 (m, 4H), 3.72-3.62 (m, 4H), 3.32 (s, 6H). C NMR (100 MHz, DMSO-d$_6$) $\delta$ 158.0, 154.6, 148.8, 139.5, 133.1, 128.5, 123.8, 123.2, 122.7, 114.8, 70.4, 69.9, 19.2 ppm.

Example 16: Quinuclidin-3-yl[(2-4′-(3-methoxypropoxy)-[1,1′-biphenyl]-4-yl)propanoic acid (Compound 16)]

To a stirred solution of 4-iodophenol (10.05 g), NaI (50.2 mmol) and 1-chloro-3-methoxypropane (6.4 mL, 1L, 57.1 mmol), the mixture was heated at reflux overnight and then concentrated. The residue was taken up in water and extracted with ethyl acetate. The combined extracts were washed with aqueous sodium bicarbonate solution, dried (Na$_2$SO$_4$) and concentrated. The crude material was purified by flash chromatography over silica using hexane/ethyl acetate:ethyl acetate:hexane (1:1:1) to afford 1-iodo-4-(3-methoxypropoxy)benzene as a colourless oil (4.39 g, 33%). This intermediate and ethyl 2-methyl-2-(4-(4,4,5,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate were reacted according to General Procedure F to generate ethyl 2-(4′-(3-methoxypropoxy)-[1,1′-biphenyl]-4-yl)-2-2-methylpropanoate. To a stirred solution of this compound (0.693 g, 1.94 mmol) in 1:1:1:1 (v/v/v) tetrahydrofuran/ethanol/water (10 mL), was added lithium hydroxide monohydrate (0.326 g, 7.77 mmol). The mixture was heated at reflux overnight and then concentrated. The residue was dissolved in water, treated with 1N hydrochloric acid (10 mL) and extracted with ethyl acetate. The combined, organic layers were washed with brine, dried (Na$_2$SO$_4$) and concentrated to afford 2-(4′-(3-methoxypropoxy)-[1,1′-biphenyl]-4-yl)-2-methylpropanoic acid as a waxy, off-white solid (0.630 g, 99%). This intermediate and und quinuclidin-3-ol were reacted according to General Procedure F to generate the title compound as a glassy, colourless, solid (62%). H NMR (400 MHz, DMSO-d$_6$) $\delta$ 7.61-5.1-7.29 (m, 7H), 7.00 (d, $J$ = 8.8 Hz, 2H), 6.47-4.36 (m, 4H), 4.05 (t, $J$ = 6.4 Hz, 2H), 3.48 (t, $J$ = 6.3 Hz, 2H), 3.26 (s, 3H), 1.30-2.25 (m, 6H), 2.04-1.74 (m, 4H), 1.65-1.23 (m, 9H) ppm. C NMR (100 MHz, DMSO-d$_6$) $\delta$ 158.0, 154.5, 146.7, 137.4, 132.4, 127.5, 125.7, 125.2, 114.8, 69.9, 68.5, 64.6, 57.9, 55.4, 54.2, 46.9, 46.0, 29.4, 29.0, 25.2, 24.1, 19.2 ppm.
ppm. Purity: 97.7%, 98.2% (210 & 254 nm) UPLCMS; retention time: 0.96 min; (M+H')^+

Example 17: Quinuclidin-3-yl(2-(4'-(hydroxymethyl)-[1,1'-biphenyl]-4-yl)propan-2-yl)carbamate (Compound 17)

Using General Procedure E and the reaction inputs ethyl 2-(4-bromophenyl)-2-methylpropanoate and 4-formylphenylboronic acid, ethyl 2-(4'-formyl-[1,1'-biphenyl]-4-yl)-2-methylpropanoate was prepared as a pale amber solid. This intermediate and quinuclidin-3-ol were reacted according to General Procedure F to generate quinuclidin-3-yl(2-(4'-formyl-[1,1'-biphenyl]-4-yl)propan-2-yl)carbamate as a foamy, yellow solid. To a stirred solution of this material (0.755 g, 1.92 mmol) in 2:1 (v/v) tetrahydrofuran/ethanol (15 mL) was added sodium borohydride (0.073 g, 1.93 mmol). After 45 minutes, the reaction was diluted with water and extracted with chloroform. The combined extracts were dried (Na$_2$SO$_4$) and concentrated onto silica. Flash chromatography over silica using a chloroform/methanol/ammonia eluent provided the title compound as a white solid (0.323 g, 43%). 

$^1$H NMR (400 MHz, DMSO-d$_6$) δ 7.66-7.29 (m, 9H), 5.18 (t, J = 5.7 Hz, 1H), 4.53 (d, J = 5.7 Hz, 2H), 4.46-4.37 (m, 1H), 3.11-2.19 (m, 6H), 2.11-1.10 (m, 11H) ppm. $^13$C NMR (100 MHz, DMSO-d$_6$) δ 154.7, 147.3, 141.5, 138.4, 137.7, 127.0, 126.2, 126.1, 125.3, 120.6, 62.6, 55.4, 54.2, 46.9, 45.9, 29.4, 24.2, 22.2 ppm. Purity: 97.5%, 99.1% (210 & 254 nm) UPLCMS; retention time: 0.73 min; (M+H')^+ 395.

Example 18: Quinuclidin-3-yl(2-(4'-(2-hydroxyethyl)-[1,1'-biphenyl]-4-yl)propan-2-yl)carbamate (Compound 18)

Using General Procedure E and the reaction inputs 1-(2-(benzyloxy)ethyl)-4-bromobenzene and ethyl 2-methyl-2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate, ethyl 2-(4'-(2-(benzyloxy)ethyl)-[1,1'-biphenyl]-4-yl)-2-methylpropanoate was prepared as a colourless gum. To a stirred solution of this compound (1.34 g, 3.33 mmol) in 1:1:1 (v/v/v) tetrahydrofuran/ethanol/water (18 mL) L was added lithium hydroxide monohydrate (0.698 g, 16.6 mmol). After heating at reflux overnight, the reaction was concentrated, and partitioned between water and diethyl ether. The resulting emulsion was extracted repeatedly with 0.2 N aqueous sodium hydroxide.
solution (5 x 50 mL). The clear portion of the aqueous layer was removed each time. The combined aqueous layers were treated with 1.0 N hydrochloric acid (80 mL) and the resulting suspension of white solid was extracted with ethyl acetate. The combined organic layers were dried (Na$_2$SO$_4$) and concentrated to afford 2-(4’-(2-(benzyloxy)ethyl)-1-[1,1’-biphenyl]-4-yl)-2-methylpropanoic acid as a white solid (1.20 g, 96%). This his compound and quinuclidin-3-ol were reacted according to General Procedure F to generate quinuclidin-3-yl (2-(4’-(2-benzyloxyethyl)-[1,1’-biphenyl]-4-yl)propan-2-yl)carbamate. To a stirred solution of this material (0.435 g, 0.806 mmol) in methanol 10 mL 0.087 g). The mixture was cycled between vacuum and a nitrogen purge several times, es, and refilling with hydrogen after the last evacuation. After 1.25 hours the reaction was filtered and concentrated through Celite and concentrated. The residue was taken up in aqueous sodium carbonate solution and extracted with 4:1 (v/v) chloroform/isopropanol. The combined extracts were dried (Na$_2$SO$_4$) and concentrated onto silica. Flash chromatography over silica using a chloroform/methanol/ammonia gradient provided the purified title compound as a colourless solid. ¹H NMR (400 MHz, DMSO-d$_6$) δ 7.85-7.63 (m, 1H), 7.63-7.19 (m, 8H), 4.78-4.62 (m, 2H), 3.71-2.78 (m, 8H), 2.76 (t, J = 6.8 Hz, 2H, 12.26-1.96 (m, 2H), 1.96-1.40 (m, 9H) ppm. ¹³C NMR (100 MHz, DMSO-d$_6$) δ 153.8, 146.8, 138.7, 137.9, 137.6, 129.4, 126.3, 126.1, 125.3, 66.2, 66.2, 154.4, 152.8, 84.5, 44.5, 38.6, 29.5, 29.2, 24.0, 19.9, 19.9, 16.6 ppm. Purity: 100%, 100% (210 & 254 nm) UPLCMS; retention time: 0.75 min; (M+H)$^+$ 409. Example 19: Quinuclidin-3-yl (2-(2-(4-(3-methoxypropoxy)phenyl)thiazol-4-yl)propan-2-yl)carbamate (Compound 19)

To a stirred suspension of 4-methoxythiobenzamide (9.99 g, 59.7 mmol) in ethanol 10 mL (75 mL), was added ethyl 4-chloroacetoacetate (8.1 mL, 60 mmol). The mixture was, as heated at reflux for 4 hours before cooling, adding additional ethyl 4-chloroacetoacetate (0.81 mL, 6.0 mmol) and returning to reflux. After 4 more hours of heating, the reaction was concentrated and partitioned between ethyl acetate and aqueous sodium bicarbonate solution. The organic layer was combined with additional ethyl acetate extracts, dried (Na$_2$SO$_4$), and concentrated. The crude product was purified by flash chromatography over silica using a hexane/ethyl acetate gradient to afford ethyl 2-(2-(4’-4, 4-...)
methoxyphenyl)thiazol-4-yl)aceta to pale amber oil (14.51 g, 87%). To a stirred solution of this compound (14.48 g, 52.2 mmol) in N,N-dimethylformamide (125 mL) was added sodium hydride (60% dispersion in mineral oil; 6.27 g, 157 mmol), portionwise over 15 minutes. The resulting red suspension was cooled (0 °C) and treated, dropwise over 10 minutes, with iodomethane (9.80 mL, 157 mmol). The cooling bath was allowed to stir for 4 hours before concentrating and then partitioning. The residue between ethyl acetate and water. The organic layer was washed twice with water, dried (Na₂SO₄), and concentrated. The residue was purified by flash chromatography over silica using a hexane/ethyl acetate gradient to afford ethyl 2-(2-(4-methoxyphenyl)thiazol-4-yl)-2-methylpropanoate as a pale amber oil (14.12 g, 89%). To a stirred solution of this intermediate (14.12 g, 46.24 mmol) in methylene chloride (250 mL) was added boron tribromide (11.0 mL, 116 mmol), dropwise over 5 or 5 minutes. After stirring overnight, the reaction was quenched by the slow addition of methanol (~20 mL) and then concentrated. The residue was taken up in methanol (250/50 mL) and concentrated sulfuric acid (7.0 mL). The stirred solution was heated at reflux for 2 hours, concentrated, and partitioned between ethyl acetate and aqueous sodium bicarbonate solution. The organic layer was combined with the second ethyl acetate extract of the aqueous layer, dried (Na₂SO₄), and concentrated to afford methyl 2-(2-(4-(4-hydroxyphenyl)thiazol-4-yl)-2-methylpropanoate as a white solid (12.56 g, 98%). To an aq stirred solution of 1-bromo-3-methoxypropane (1.66 g, 10.8 mmol) in acetone (30 mL) was added phenol intermediate (2.00 g, 7.21 mmol) and potassium carbonate (1.25 g, 9.04 mmol). The mixture was heated overnight at reflux, filtered, and concentrated. The residue was purified by flash chromatography over silica using a hexane/ethyl acetate gradient to afford methyl 2-(2-(4-(3-methoxypropoxy)phenyl)thiazol-4-yl)-2-methylpropanoate as a white solid (2.47 g, 98%). To a stirred solution of this intermediate (2.45 g, 7.01 mmol) in 1:1:1 (v/v/v) tetrahydrofuran/ethanol/water (45 mL) was added lithium hydroxide monohydrate (1.47 g, 35.0 mmol). After overnight stirring, the reaction was concentrated and partitioned between water and diethyl ether. The aqueous layer was treated with 1.0 N hydrochloric acid (40 mL) and extracted with ethyl acetate. The combined extracts were dried (Na₂SO₄) and concentrated to afford 2-(2-(4-(3-methoxypropoxy)phenyl)thiazol-4-yl)-2-methylpropanoic acid as a white solid (2.19 g, 40.93%). This compound and quinuclidin-3-ol were reacted according to General ral...
Procedure: To generate the title compound as a soft, faint amber solid, 1H NMR (400 MHz, DMSO-d6) δ 7.82 (d, J = 8.9 Hz, 2H), 7.36 (brs, 1H), 7.24 (brs, 1H), 7.03 (d, J = 8.9 Hz, 2H), 4.49-4.41 (m, 1H), 4.07 (t, J = 6.4 Hz, 2H), 3.48 (t, J = 6.4 Hz, 2H), 3.26 (s, 5H), 3.09-2.26 (m, 6H), 2.02-1.91 (m, 2H), 1.91-1.03 (m, 11H) ppm. Retention time: 0.87 min; t(M+H)+ 460.10.

Example 20: Quinuclidin-3-yl(2-(2-(4-(2-methoxyethoxy)phenyl)thiazol-4-yl)propan-2-yl)carbamate (Compound 20)

To a stirred solution of 2-bromoethyl methyl ether (1.88 g, 13.5 mmol) in acetonitrile was added methyl 2-(2-(4-hydroxyphenyl)thiazol-4-yl)-2-methylpropanoate (prepared as described in Example 19, 2.00 g, 7.21 mmol) and potassium carbonate (1.56 g, 11.31 mmol). After heating at reflux overnight, the mixture was treated with additional 2-bromoethyl methyl ether (1.88 g, 13.5 mmol) and potassium carbonate (1.56 g, 11.31 mmol). The reaction was heated at reflux for a second night, filtered and concentrated. The residue was purified by flash chromatography over silica using a hexane/ethyl acetate gradient to afford methyl 2-(2-(4-(2-methoxyethoxy)phenyl)thiazol-4-yl)-2-methylpropanoate as a white solid (2.71 g, 90%). To a stirred solution of this compound (2.71 g, 8.08 mmol) in 1:1:1 (v/v/v) tetrahydrofuran/ethanol/water (50 mL) was added lithium hydroxide monohydrate (1.70 g, 40.5 mmol). After overnight stirring, the reaction was concentrated and partitioned between water and diethyl ether. The aqueous layer was treated with 1.01 M N hydrochloric acid (41 mL) and extracted with ethyl acetate. The combined extracts were dried (Na2SO4) and concentrated to afford 2-(2-(4-(2-methoxyethoxy)phenyl)thiazol-4-yl)-2-methylpropanoic acid as a white solid (2.57 g, 99%). This compound and quinuclidin-3-ol were reacted according to General Procedure F to generate the title compound as a pale amber solid. 1H NMR (400 MHz, DMSO-d6) δ 7.82 (d, J = 8.9 Hz, 2H), 7.36 (brs, 1H), 7.24 (brs, 1H), 7.04 (d, J = 8.8 Hz, 2H), 4.49-4.41 (m, 1H), 4.19-4.12 (m, 2H), 3.71-3.65 (m, 2H), 3.32 (s, 3H), 3.11 (d, J = 13.5 Hz, 2H), 2.86-2.19 (m, 5H), 1.92-1.16 (m, 11H) ppm. Retention time: 0.97 min; t(M+H)+ 460.10. C NMR (100 MHz, DMSO-d6) δ 165.7, 159.9, 154.6, 127.5, 126.2, 114.9, 112.2, 70.3, 70.1, 67.1, 58.2, 55.4, 53.5, 46.9, 99%.
Example 21: Quinuclidin-3-yl 2-(5-(4-(2-methoxyethoxy)phenyl)pyridin-2-yl)propan-2-yl carbamate (Compound 21)

Using General Procedure E and the reaction inputs 5-bromopicolinonitrile and 2-(4-(2-(2-methoxyethoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 5-(4-(2-(2-methoxyethoxy)phenyl)picolinonitrile was prepared. Cerium(III)trichloride (8.05 g, 21.61 mmol) was loaded into a flask and dried by heating (170°C) under vacuum for 3 hours. The solid was taken up in tetrahydrofuran (20 mL) and stirred vigorously for 30 minutes. The suspension was cooled to -78°C and treated, dropwise, with a 3.0 M solution of methyl lithium in diethyl ether (7.2 mL, 21.6 mmol). Following addition, the reaction was stirred at -78°C for 1 hour before adding a solution of the above arylborate (1.83 g, 7.20 mmol) in tetrahydrofuran (20 mL). The mixture was maintained at -78°C for 2 hours and then allowed to warm to room temperature. At this time, the reaction was quenched by the addition of aqueous ammonium hydroxide (10 mL) and filtered through a plug of Celite. The filtrate was extracted with ethyl acetate and the combined extracts were washed with brine, dried with Na₂SO₄, and concentrated. The residue was purified by flash chromatography over silica gel to afford 2-(5-(4-(2-methoxyethoxy)phenyl)pyridin-2-yl)propan-2-amine as a yellow solid (0.800 g, 39%).

To a stirred suspension of this intermediate (0.500 g, 1.75 mmol) in water (10 mL) and concentrated hydrochloric acid (0.44 mL) was added toluene (10 mL). The mixture was cooled to 0°C and treated with simultaneous over 1 hour, solutions of triphosgene (0.776 g, 2.62 mmol) in toluene (10 mL) and sodium bicarbonate (2.2 g, 26 mmol) in water (20 mL). Following the additions, the reaction was stirred for an additional 30-60 minutes, before the upper toluene layer was removed and dried (Na₂SO₄). At the same time, a stirred solution of quinuclidin-3-ol (0.445 g, 3.64 mmol) in tetrahydrofuran (10 mL) was treated with sodium hydride (60% dispersion in mineral oil; 0.154 g, 3.85 mmol). This mixture was stirred for 5 minutes and then added to the solution of crude diisocyanate in toluene. The reaction was stirred for 10 minutes, quenched with the addition of brine (5 mL) and extracted with ethyl acetate. The combined extracts were dried with Na₂SO₄ and concentrated. The residue was purified by flash chromatography over
reversed phase silica to afford the title compound as a light yellow solid (0.100 g, 13%).

\[ \text{H NMR (}500\text{MHz, CDCl}_3) \delta 8.70-8.70 (d, J = 2.0\text{Hz}, 1H), 7.83-7.81 (m, 1H), 7.49-7.47 (d, J = 9.0\text{Hz}, 2H), 7.43-7.43 (d, J = 8.0\text{Hz}, 1H), 7.03-7.01 (d, J = 8.5\text{Hz}, 2H), 6.63-6.63 (br's, 1H), 4.68-4.66 (m, 1H), 4.16 (t, J = 5.0\text{Hz}, 2H), 3.77 (t, J = 5.0\text{Hz}, 2H), 3.45 (s, 5H), 3.19-2.70 (m, 6H), 2.15-1.89 (m, 12H), 1.76 (s, 6H), 1.73-1.36 (m, 3H) ppm. \]

\[ \text{C NMR (}100\text{MHz, DMSO-d}_6) \delta 157.7, 154.5, 146.8, 137.4, 132.7, 128.4, 128.1, 127.9, 127.4, 71.0, 59.5, 55.7, 46.4, 27.9, 25.4, 24.6, 19.5 ppm. \]

Purity: >99% (214 & 254 nm) LCMS; retention time: 1.32 min; (M+H\(^+\)) 440.2. 2.

Example 22: Quinuclidin-3-yl (2-(4'-cyanopropanyloxy)-[1,1'-biphenyl]-4-yl)propan-2-yl carbamate (Compound 22)

To a stirred solution of 4-bromophenol (17.1 g, 98.8 mmol) in acetonitrile (150 mL) was added 1-bromobutyl nitrite (12.3 mL, 124 mmol) and potassium carbonate (15.0 g, 109.09 mmol). The mixture was heated to reflux overnight, cooled and concentrated. The residue was taken up in water and extracted with ethyl acetate. The combined extracts were dried (Na\(_2\)SO\(_4\)) and concentrated; the crude material was purified by flash chromatography (dichloromethane/ethyl acetate as a white solid (20.8 g, 88%). To a stirred solution of this product in N,N,N-dimethylformamide (100 mL), was added bis(pinacolato)diboron (4.60 g, 18.1 mmol), potassium acetate (7.41 g, 75.5 mmol) and [1,1'-bis(diphenylphosphino)ferrocene]-dichloropalladium(II) complex with dichloromethane (0.616 g, 1.04 mmol). The mixture was heated to reflux overnight and then concentrated. The residue was taken up in ethyl acetate and washed with water and brine. The organic layer was dried (Na\(_2\)SO\(_4\)) and concentrated; the crude product was purified by flash chromatography over silica gel using a hexane/ethyl acetate eluent to afford 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)butanenitrile as a white solid (3.43 g, 79%). This product and the quinuclidin-3-yl(2-(4-bromophenyl)propan-2-yl) carbamate (prepared by reacting 4-quinoledin-3-ol and 2-(4-bromophenyl)propan-2-amine using General Procedure F) were reacted according to General Procedure E to generate the title compound as a white solid. 1H NMR (400 MHz, DMSO-d\(_6\)) \[ \delta 7.67-7.72 (m, 7H), 7.02 (d, J = 8.8\text{Hz}, 2H), 4.50-4.33 (3 \text{H}, 1H), 4.08 (t, J = 6.0\text{Hz}, 2H), 3.14-2.18 (m, 8H), 2.04 (quin, J = 6.7\text{Hz}, 2H), 1.94-1.44-1.70 (m, 11H) ppm. \]

13C NMR (100 MHz, DMSO-d\(_6\)) \[ \delta 157.7, 154.5, 146.8, 137.4, 132.7, 7, \]

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127.6, 125.7, 125.2, 120.2, 114.9, 70.0, 65.8, 55.4, 54.2, 46.9, 45.9, 29.4, 25.3, 24.7, 24.2, 19.2, 13.4 ppm. Purity: 100%.

Example 23: Quinuclidin-3-yl (2-(4'-(cyanomethoxy)-[1,1'-biphenyl]-4-yl)propan-2-yl)carbamate (Compound 23)

Using General Procedure E and the reaction inputs quinuclidin-3-yl (2-(4-bromophenyl)propan-2-yl)carbamate (prepared by reacting quinuclidin-3-ol and 2-(4-bromophenyl)propan-2-amine using General Procedure F) and 4-(cyanomethoxy)phenylboronic acid, the title compound was prepared as a pale amber solid. 

$^1$H NMR (400 MHz, DMSO- $d_6$) €7.65 (d, $J=8.2$ Hz, 2H), 7.60-7.31 (m, 5H), 7.15 (d, $J=8.9$ Hz, 2H), 5.21 (s, 2H), 4.53-4.30 (m, 1H), 3.18-2.19 (m, 6H), 2.05-1.18 (m, 11H) ppm.

$^{13}$C NMR (100 MHz, DMSO- $d_6$) €155.8, 154.6, 147.2, 137.2, 134.4, 127.8, 8, 126.0, 125.3, 116.7, 115.3, 70.0, 55.4, 54.2, 53.5, 54.9, 45.9, 29.4, 25.2, 24.2, 19.2 ppm. Purity: 100%.

Example 24: Tissue distribution of Compound 1 in a mouse model of proteinopathies

A mouse model has been described (Gba1 $^{D409V/D409V}$) that exhibits progressive accumulation of proteinase K-resistant D-synuclein, ubiquitin and tau aggregates in the central nervous system. This is reminiscent of the protein deposits seen, for example, in Lewy neurites in patients with Parkinson’s disease and Lewy body dementia. These mice also display a demonstrable hippocampal memory deficit. The distribution of in brain and liver tissue of these mice was investigated following oral administration of Compound 1.

Methods:

Gba1 $^{D409V/D409V}$ mice (harboring a point mutation at residue 409 in the murine Gba1 gene) were bred under a protocol approved by the Institutional Animal Care and Use Committee. Treatments were administered as described and the animals were humanely ely sacrificed at pre-determined time points or upon reaching a humane endpoint. A subset of Gba1 $^{D409V/D409V}$ mice received Compound 1 administered in food using a formulation calculated to provide 60 mg/kg/day. Drug administration was initiated when...
pups were weaned at 4 weeks of age and continued until euthanasia at 4 or 10 months of age. The concentration of Compound 1 in brain and liver tissues was determined by mass spectrometry (see e.g. Ramanathan et al., “The emergence of high-resolution MS as the premier analytical tool in the pharmaceutical bioanalysis arena” Bioanalysis. Mar 2012;4(5):467-469).

Results:

Mice fed with a diet containing Compound 1 demonstrated tissue exposure of 217 ± 12 ng/g tissue in the cortex; and 10512 ± 603 ng/g tissue in the liver, i.e. the concentration of Compound 1 in the brain was approximately 2% of the concentration in the liver. These results demonstrate that Compound 1 crosses the blood-brain barrier.

Example 25: Administration of Compound 1 improves memory deficit in Gba1 D409V/D409V mice.

The extent of memory deficit in Gba1 D409V/D409V mice was evaluated using novel object recognition (NOR) and fear conditioning (FC) tests.

Methods:

Mice were bred and fed with the Compound 1 diet as described in Example 24.24.

In the NOR test, four month-old wild type (WT) and Gba1 D409V/D409V mice were dosed with Compound 1 starting at 4 weeks of age and were subjected to the NOR test at 3 or 3 months (2 months post-treatment). Mice were individually habituated to explore an open-field box for 5 minutes. During the first training session (T1), two identical objects were symmetrically placed into the open-field; 14 inches from each other. Animals were allowed to explore for 5 minutes. The number of investigations was recorded by a blinded investigator. After a 24-hour retention period, animals were tested (T2) for their recognition of a novel object. Mice were placed back into the open-field box for 5 minutes, and the time spent investigating the familiar and novel objects was recorded.
Statistical analyses were performed by Student’s t-test or analysis of variance (ANOVA) followed by Newman-Keuls’ post-hoc test. Preference for novelty was defined as investigating the novel object more than 50% of the time by a one-sample t-test. All statistical analyses were performed with GraphPad Prism 4.0 (GraphPad Software, San Diego, CA). Values of p < 0.05 were considered significant.

Ten month-old wild-type (WT) and Gba1<sup>H409D</sup> mice were subjected to fear memory tests. For the fear conditioning tests, mice were trained in four of the eight chambers. Near-Infrared<sup>®</sup> Fear Conditioning System chambers (St. Albans, VT). Mice were placed in the contextual fear chamber in “Context A,” which consists of a lighting, a neutral background and a stainless steel grid floor. The mice were trained with a 3-trial delay-cued protocol as defined below. The mice were first given 2 minutes to explore the chamber in Context A before a conditioned stimulus (CS) of a 2000 Hz cue was given. Thirty seconds later, a one-second unconditioned stimulus (US) of 0.6 mA foot shock was applied. With an inter-trial interval (ITI) of 30 sec, this US-CS response was repeated three times. After a 24 hour retention period, mice were brought back to the testing room and habituated to the room for 1 hour. The mice were again placed in Context A for 5 minutes and freezing to the context was recorded. Freezing (defined as the lack of movement, except for respiration) was recorded using a near-infrared camera system. Mice were then removed from the chamber and placed back into their respective cages. After 1 hour, the mice were placed back into the chamber in a novel context, Context B. The mice were allowed to explore the cage for 3 minutes in the novel environment, followed by 3 minutes of the 3-Tone auditory cue with the same ITI as the training protocol. Again, freezing to the novel environment and the cue were assessed with the near-infrared camera system. Contextual fear memory is defined as the freezing from the training context minus the freezing in the novel context. Cued memory is defined as the freezing to the cue in the novel context.

Results:

Results are of the NOR test are shown in Figure 1. Results are expressed as percentages of target investigations during training (T1) or testing (T2).

In detail, trial 1 (training, solid bars) revealed no object preference when exposed to two similar objects. After a 24 hour retention period, mice were presented with a novel object.
In trial 2 (testing, hatched bars), WT mice investigated the novel object significantly more frequently ($p < 0.05$). In contrast, untreated Gba1$^{D409V/D409V}$ mice (middle, hatched bar) showed no preference for the novel object, indicating a cognitive impairment. Untreated Gba1$^{D409V/D409V}$ mice showed no preference for the novel object, indicating a cognitive impairment. Control Gba1$^{D409V/D409V}$ mice (middle, hatched bars) showed decreased freezing responses in contextual and cued FC testing, confirming the memory impairment. Treatment with Compound 1 (right-hand, solid bars) improved the freezing responses in the contextual paradigm (Fig. 2A), indicating an improved hippocampal memory. On the other hand, administration of Compound 1 had no effect on cued memories (Fig. 2B), suggesting the amygdala fear responses are not affected by quinuclidine compounds as described herein.

Example 26: Administration of Compound 1 improves memory deficit in mice overexpressing A53T D-synuclein.

The extent of memory deficit was evaluated in mice overexpressing A53T D-synuclein using novel object recognition (NOR) and fear conditioning (FC) tests. These mice develop α-synuclein inclusions similar to those observed in humans suffering from α-synucleinopathies, and present with neurodegeneration and severe motor impairment.

Methods;

PrP-A53T-SNCA transgenic mice ("A53T" mice) express human A53T α-synuclein (line M83), under the transcriptional control of the murine PrP promoter (Giasson et al., 2002).

The NOR test was performed as described in Example 25, except that the mice were dosed with Compound 1 at 6 weeks of age and were subjected to the NOR test at 4.5 months.

The FC test was also performed as described in Example 25, except that the mice were subjected to the test at 8 months of age.

Results:

Results are of the NOR test are shown in Figure 3. Results are expressed as percentages of target investigations during testing (T2). During training, animals revealed no object preference when exposed to two similar objects (data not shown). I.

The WT mice investigated the novel object significantly more frequently (left-hand bar, **p < 0.05**). In contrast, A53T mice showed no preference for the novel object, indicating a cognitive impairment (middle, hatched bar). A53T mice treated with Compound 1 exhibited a trend to reversal of their memory deficit when presented with the novel object during the testing trial (right-hand, solid bar). The results are represented as the means ± the SEM. The horizontal line demarcates 50% target investigations, which represents no preference for either object. J.

Results are of the FC tests are shown in Figure 4. Figure 4A shows the results relating to contextual memory. Figure 4B shows the results relating to cued memory. Control A53T mice (middle, hatched bars) showed decreased freezing responses in contextual and cued FC testing, confirming the memory impairment. Treatment with Compound 1 improved the freezing responses in the contextual paradigm (Fig. 4A, right-hand, solid bar), indicating an improved hippocampal memory. Administration of Compound 1 had only a marginal effect on cued memories (Fig. 4B, right-hand, solid bar), suggesting that amygdala fear responses are not affected by administration of quinuclidine compounds as described herein. Without wishing to be bound by theory, the inventors postulate that the effects on memory which are observed in these mouse models may be due to a reduction in protein aggregates within neural tissue of the mice concomitant with a reduction in levels of toxic substrates within those cells.
compounds as described herein are capable of, for example, reducing intra-cellular levels of sphingolipids which may have an adverse impact on neural tissue).  

Example 27: Administration of Compound 1 reduces protein aggregation in the brain.  

The ability of quinuclidine compounds as described herein to reduce and/or reverse protein aggregation in the brains of Gba1<sup>D409V/D409V</sup> mice was assessed.

**Methods:**

Wild-type (WT) and Gba1<sup>D409V/D409V</sup> mice were fed the control diet or the Compound 1 diet as described in Example 24. The accumulation of proteins (ubiquitin, D-synuclein, and protein tau) was determined by hippocampal quantification and protein immuno-reactivity both with and without treatment with Compound 1. Protein levels at 4 and 40 weeks of age in Gba1<sup>D409V/D409V</sup> mice were used as baseline levels; protein levels were measured at 16 and at 40 weeks of age.

For histological analysis, mice were perfused with cold PBS. Brains were removed and post-fixed in 10% (v/v) neutral-buffered formalin for 48 hours. Tissues were then placed in 30% sucrose, embedded, and sectioned at 20 µm in a cryostat. Some tissues were pretreated with proteinase K (1:4 dilution; DAKO, Carpinteria, CA) for 7 minutes at room temperature to expose D-synuclein and other aggregated proteins. Brain sections were blocked with 10% (v/v) serum for 1 hour at room temperature and incubated with the following antibodies: mouse anti-ubiquitin (1:500; Millipore, Billerica, MA), rabbit anti-alpha-synuclein (1:300; Sigma, St. Louis, MO), and mouse anti-tau (1:500, Tau-5, Millipore, Billerica, MA). Brain sections were then incubated for 1 hour at with either a donkey anti-mouse AlexaFluor-488 or a donkey anti-rabbit AlexaFluor-555 secondary antibody (1:250 dilution; Invitrogen, Carlsbad, CA). For D-synuclein aggregate quantification, a cyanine<sub>3</sub>-tyramide signal amplification kit was used (PerkinElmer, Waltham, MA). Nuclei were stained with DAPI (Sigma, St. Louis, MO). Sections were cover-slipped with aqua poly/mount (Polysciences, Warrington, PA).  

For morphometric analysis, sections were imaged with a SPOT<sup>®</sup> camera (Diagnostic Instruments, Sterling Heights, MI) paired with a Nikon Eclipse E800 fluorescence microscope equipped with a 20X objective lens. The stratum radiatum, external to the...
Results:

Hippocampal quantification of ubiquitin aggregates is shown in Figures 5A (16 weeks of age, n≥5 per group) and 5B (40 weeks of age, n≥8 per group). The results are represented as the means±the SEM. Bars with different letters are significantly different from each other (p<0.05). The images in Figure 6 show ubiquitin immunoreactivity (green) in the hippocampi of 40-week-old Gba1<sup>D409V/D409V</sup> mice control (Fig. 6A) or treated with 1<sup>st</sup> Compound 1 (Fig. 6B). DAPI nuclear staining is shown in blue.

Hippocampal quantification of proteinase K-resistant α-synuclein aggregates is shown in Figures 7A (16 weeks of age, n≥5 per group) and 7B (40 weeks of age, n≥8 per group). The results are represented as the means±the SEM. Bars with different letters are significantly different from each other (p<0.05). The images in Figure 8 show proteinase K-resistant α-synuclein immunoreactivity (red) in the hippocampi of 40-week-old Gba1<sup>D409V/D409V</sup> mice control (Fig. 8A) or treated with 1<sup>st</sup> Compound 1 (Fig. 8B). DAPI nuclear staining is shown in blue.

Hippocampal quantification of protein-tau aggregates is shown in Figures 9A (16 weeks of age, n≥5 per group) and 9B (40 weeks of age, n≥8 per group). The results are represented as the means±the SEM. Bars with different letters are significantly different from each other (p<0.05). The images in Figure 10 show tau immunoreactivity (green) in the hippocampi of 40-week-old Gba1<sup>D409V/D409V</sup> mice control (Fig. 10A) or treated with 1<sup>st</sup> Compound 1 (Fig. 10B). DAPI nuclear staining is shown in blue.
The Gba1<sup>Wt/αsnn</sup> D409V/D409V mice accumulate ubiquitin, α-synuclein and protein tau aggregates from 4 to 40 weeks of age. Treatment with quinuclidine compounds as described herein, (i) blocked the accumulation of ubiquitin aggregates at 40 weeks of age, reducing its levels to wildtype controls; (ii) reduced the accumulation of α-synuclein aggregates at 40 weeks of age; and (iii) blocked the accumulation of tau aggregates at 40 weeks of age. 

The results presented herein show the effects of quinuclidine compounds as described herein on neuronal α-synuclein and protein tau processing <i>in vivo</i> and demonstrate the therapeutic potential of administering quinuclidine compounds as described herein for treating proteinopathies.

Example 28: Preparation of (S)-Quinuclidin-3-yl(2-(2-(4-fluorophenyl)thiazol-4-yl)propan-2-yl)carbamate free base

**Step 1: Dimethylation with methyl iodide**

A 3N RB flask was equipped with a thermometer, an addition funnel, and a nitrogen inlet. The flask was flushed with nitrogen and potassium tert-butoxide (MW 112.21, 75.4 mmol, 8.46 g, 4.0 equiv., white powder) was weighed out and added to the flask via a powder funnel followed by the addition of THF (60 mL). Most of the potassium tert-butoxide dissolved to give a cloudy solution. This mixture was cooled in an ice-water bath to 0-2°C (internal temperature). In a separate flask, the starting ester (MW 265.3, 18.85 mmol, 5.0 g, 1.0 equiv.) was dissolved in THF (18 mL + 2 mL as rinse) and transferred to the addition funnel. This solution was added dropwise to the cooled mixture over a period of 25-30 min, keeping the internal temperature below 5°C during the addition. The reaction mixture was cooled back to 0-2°C. In a separate flask, a solution of methyl iodide (MW 141.94, 47.13 mmol, 6.7 g, 2.5 equiv.) in THF (6 mL) was prepared and transferred to the addition funnel. The flask containing the methyl iodide solution was held...
then rinsed with THF (1.5 mL) which was then transferred to the addition funnel already containing the clear colorless solution of methyl iodide in THF. This solution was added carefully dropwise to the dark brown reaction mixture over a period of 30-40 min, in keeping the internal temperature below 10°C at all times during the addition. After the addition was complete, the slightly turbid mixture was stirred for an additional 1 h during which time the internal temperature dropped to 0-5°C. After stirring for an hour at 0-5°C, the reaction mixture was quenched with the slow dropwise addition of 5.0 M aqueous HCl (8 mL) over a period of 15-7 min. The internal temperature was maintained below 20°C during this addition. After the addition, water (14 mL) was added and the mixture was stirred for 2-3 min. The stirring was stopped and the two layers were allowed to separate. The two layers were then transferred to a 250 mL NRB flask and the THF was as evaporated in vacuo as much as possible to obtain a biphasic layer of THF/product and water. The two layers were allowed to separate. A THF solution of the Step 1 product was used in the next reaction.

Step 2: Hydrolysis of the ethyl ester with LiOH monohydrate

The crude ester in THF was added to the reaction flask. Separately, LiOH.H₂O (MW 41.96, 75.0 mmol, 3.15 grams, 2.2 equiv.), was weighed out in a 100 mL beaker to which a stir bar was added. Water (40 mL) was added and the mixture was stirred until all the solid dissolved to give a clear, colorless solution. This aqueous solution was then added to the 250 mL NRB flask containing the solution of the ester in tetrahydrofuran (THF). A condenser was attached to the neck of the flask and a nitrogen inlet was attached at the top of the condenser. The mixture was heated at reflux for 16 h, after which the mixture was cooled to room temperature. The THF was then evaporated in vacuo to obtain a brown solution. An aliquot of the brown aqueous solution was analyzed by HPLC and LC/MS for complete hydrolysis of the ethyl ester. Water (15 mL) was added and this aqueous basic solution was extracted with TBME (2 x 40 mL) to
remove the t-butyl ester. The aqueous basic layer was cooled in an ice-water bath to 0-10°C and acidified with dropwise addition of concentrated HCl (pH 1) with stirring. To this gummy solid in the aqueous acidic solution was added TBME (60 mL) and the mixture was shaken and then stirred vigorously to dissolve all the acid into the TBME/4E layer. The two layers were transferred to a separatory funnel and the TBME layer was as separated out. The pale yellow aqueous acidic solution was re-extracted with TBME (40:40 mL) and the TBME layer was separated and combined with the previous TBME layer. The aqueous acidic layer was discarded. The combined TBME layers were dried over anhydrous Na₂SO₄, filtered, and evaporated in vacuo to remove TBME and obtain the crude acid as an orange/dark yellow oil that solidified under high vacuum to a dirty yellow colored solid. The crude acid was weighed out and crystallized by heating it in heptane/TBME (3:1, 55 mL/g crude) to give the acid as a yellow solid.

**Step 3: Formation of hydroxamic acid with NH₂OH.HCl**

![Chemical structure](image)

**Chemical Formula:** C₁₃H₁₂FNO₂S

**Exact Mass:** 265.06

**Molecular Weight:** 265.30

The carboxylic acid (MW 265.3, 18.85 mmol, 5.0 equiv.) was weighed and transferred to a 25 mL 1N RB flask under nitrogen. THF (5.0 mL) was added and the acid CID readily dissolved to give a clear, dark yellow to brown, solution. The solution was cooled to 0-2°C (bath temperature) in an ice-bath and N,N’-carbonyldiimidazole (CDI; MW 162.15, 20.74 mmol, 3.36 equiv.) was added slowly in small portions over a period of 10-15 minutes. The ice-bath was removed, and the solution was stirred at room temperature for 1 h. After 1 h of stirring, the solution was again cooled in an ice-water bath to 0-2°C (bath temperature). Hydroxylamine hydrochloride (NH₂OH.HCl; MW 69.49, 37.7 mmol, 2.62 equiv. 2.0 equiv.) was added slowly in small portions as a solid over a period of 3-5 minutes as this addition was exothermic. After the addition was complete, 10 mL water (1.0 mL) was added to the heterogeneous mixture dropwise over a period of 2-5 minutes, and the reaction mixture was stirred at 0-10°C in the ice-water bath for 5 minutes. The cooling bath was removed and the reaction mixture was stirred under
nitrogen at room temperature overnight for 20-22 h. The solution became clear as all of the \( \text{NH}_2\text{OH}.\text{HCl} \) dissolved. After 20-22 h, an aliquot of the reaction mixture was analyzed by High Pressure Liquid Chromatography (HPLC). The THF was then evaporated in vacuo and the residue was taken up in dichloromethane (120 mL) and water (60 mL). The mixture was transferred to a separatory funnel where it was shaken and the two layers allowed to separate. The water layer was discarded and the dichloromethane layer was washed with 1 N hydrochloride (HCl; 60 mL). The acid layer was discarded. The dichloromethane layer was dried over anhydrous \( \text{Na}_2\text{SO}_4 \), filtered and the solvent evaporated in vacuo to obtain the crude hydroxamic acid as a pale yellow solid that was dried under high vacuum overnight.

**Step 3 continued:** Conversion of hydroxamic acid to cyclic intermediate (not yet isolated)

- The crude hydroxamic acid (MW: 280.32, 5.1 g) was transferred to a 250 mL 1 N RB flask with a nitrogen inlet. A stir bar was added, followed by the addition of acetonitrile (50-50 mL). The solid was insoluble in acetonitrile. The yellow heterogeneous mixture was stirred for 2-3 minutes under nitrogen and CDI (MW: 162.15, 20.74 mmol, 3.36 g, 1.11 equiv.) was added in a single portion at room temperature. No exotherm was observed. The solid immediately dissolved and the clear yellow solution was stirred at room temperature for 2-2.5 h. After 2-2.5 h, an aliquot was analyzed by HPLC and LC/MS/MS which showed conversion of the hydroxamic acid to the desired cyclic intermediate. The acetonitrile was then evaporated in vacuo to give the crude cyclic intermediate as a reddish thick oil. The oil was taken up in toluene (60 mL) and the reddish mixture was heated to reflux for 2 hours during which time the cyclic intermediate released \( \text{CO}_2 \) and rearranged to the isocyanate (see below).
Step 3 continued: Conversion of the isocyanate to the free base

The reaction mixture was cooled to 50-60°C and (S)-(+)-quiniuclidinol (MW 127.18, 28.28 mmol, 3.6 g, 1.5 equiv.) was added to the mixture as a solid in a single portion. The reaction mixture was re-heated to reflux for 18 h. After 18 h, an aliquot was analyzed by HPLC/MS, which showed complete conversion of the isocyanate to the desired product. The reaction mixture was transferred to a separatory funnel and toluene (25 mL) was added. The mixture was washed with water (2 x 40 mL) and the water layers were separated. The combined water layers were re-extracted with toluene (30 mL) and the water layer was discarded. The combined toluene layers were transferred to a 500 mL Erlenmeyer flask equipped with a stir bar. This stir bar was adjusted to pH 10-12 by the dropwise addition of 50% w/w aqueous NaOH. The desired free base precipitated out of solution as a dirty yellow/gummy solid which could trap the stir bar. To this mixture was added isopropyl acetate (100 mL) and the mixture was stirred vigorously for 5 minutes. When the gummy solid went into isopropyl acetate, the stirring was stopped and the two layers were allowed to separate. The yellow isopropyl acetate layer was separated and the basic aqueous layer was re-extracted with isopropyl acetate (30 mL). The basic aqueous layer was discarded and the combined isopropyl acetate layers were dried over anhydrous Na$_2$SO$_4$, filtered into a pre-weighed RB flask, and the solvent evaporated in vacuo to
obtain the crude free base as a beige to tan solid that was dried under high vacuum overnight.

**Step 3 continued**: Recrystallization of the crude free base

The beige to tan colored crude free base was weighed and recrystallized from heptane/isopropyl acetate (3:1, 9.0 mL of solvent/g of crude free base). The appropriate amount of heptane/isopropyl acetate was added to the crude free base along with stirring bar and the mixture was heated to reflux for 10 min (free base was initially partially soluble but dissolved to give a clear reddish orange solution when heated to reflux). The heated source was removed and the mixture was allowed to cool to room temperature with stirring when a white precipitate formed. After stirring at room temperature for 3-4 h, the precipitate was filtered off under a hood under vacuum using a Buchner funnel, washed with 10 mL of heptane (20 mL) and dried under a hood under vacuum on the Buchner funnel overnight. The precipitate was then transferred to a crystallizing dish and dried at 55°C overnight in an oven. ¹H NMR (400 MHz, CDCl₃) δ 7.83 (m, 2H), 7.20 (m, 9H), 6.99 (m, 3H), 5.53 (s, 1H), 4.73 (d, J = 14.5, 8.4 Hz, 1H), 4.05 (d, J = 2.19 (m, 5H), 2.0–1.76 (m, 11H) ppm. ¹³C NMR (100 MHz, CDCl₃) δ 166.38, 165.02, 162.54, 162.8, 155.0, 130.06, 128.43, 128.34, 116.01, 115.79, 112.46, 71.18, 55.70, 54.13, 47.42, 46.52, 27.94, 25.41, 24.67, 19.58 ppm.

Example 29: Preparation of crystalline forms of (>)-Quinuclidin-3-yl(2-(2-(4-fluorophenyl)thiazol-4-yl)propan-2-yl)carbamate salts

Crystalline salts, of (>)-Quinuclidin-3-yl(2-(2-(4-fluorophenyl)thiazol-4-yl)propan-2-yl)carbamate, may be formed from the free base, prepared as described in Example 28. For example, the free base of (>)-Quinuclidin-3-yl(2-(2-(4-fluorophenyl)thiazol-4-yl)propan-2-yl)carbamate (about 50 mmol) is dissolved in IPA (140 mL) at room temperature and filtered. The filtrate is added into a 1 L r.b. flask, which is equipped with an overhead stirrer and nitrogen in/outlet. L-malic acid (about 50 mmol) is dissolved in IPA (100 mL) at room temperature and filtered. The filtrate is added into the above, 1 L r.b. liter flask. The resulting solution is stirred at room temperature (with or without seeding) for 4 to 24 hours. During this period of time, crystals form. The product is...
collected by filtration and washed with a small amount of IPA (30 ml). The crystalline solid is dried in a vacuum oven at 55 °C for 72 hours to yield the desired malate salt. 

Crystal forms of other salts, e.g., acid addition salts with succinic acid or HCl, may also be prepared in an analogous manner. 

Example 30: Administration of Compound 1 affects the subcellular localization of α-synuclein in the brain of A53T mice 

The ability of quinuclidine compounds as described herein to affect the subcellular localization of α-synuclein in the brains of A53T mice was assessed. 

Methods: 

PrP-A53T-SNCA transgenic mice (“A53T” mice) were bred and treated as described in Example 26, being dosed with Compound 1 starting at 6 weeks of age until euthanasia at 8 months of age. Mice were fed the control diet or the Compound 1 diet as described in Example 24. 

Cortical tissue homogenates from control and treated A53T mice were subjected to serial fractionation to separate soluble cytosolic (Tris-soluble), membrane-associated (Triton-soluble), and insoluble cytosolic (SDS-soluble) α-synuclein. The concentration of each fraction was quantified using the human α-synuclein ELISA kit (Biolegend, San Diego, CA). Protein concentrations were determined using the microBCA assay (Thermo Scientific Pierce, Waltham, MA). 

Results: 

Quantification of α-synuclein in the different fractions is shown in Figure 11A. A small increase in the average level of cytosolic soluble α-synuclein was observed in mice treated with Compound 1 (Fig. 11A; left-hand bar shows untreated control mice, right-hand bar shows treated mice having 114 ± 8% of the control value; n = 14, P = 0.17). The level of membrane-associated α-synuclein was significantly decreased in response to Compound 1 treatment (Fig. 11B; left-hand bar shows untreated control mice, right-hand bar shows treated mice having 75 ± 8% of the control value; n = 14, P < 0.05).
The level of insoluble α-synuclein was also significantly decreased in response to Compound 1 treatment (Fig. 11C: left-hand bar shows untreated control mice, right-hand and bar shows treated mice having 81 ± 3% of the control value, n = 14, P < 0.01). These results demonstrate that the administration of a quinuclidine compound as described herein can affect neuronal α-synuclein processing and localization in vivo and illustrate the therapeutic potential of the quinuclidine compounds described herein for treating proteinopathies.

Example 31: Administration of Compound 1 reduces protein aggregation in the brains of A53T mice.

The effect of quinuclidine compounds as described herein on protein aggregation in the brains of A53T mice was assessed.

Methods:
A53T mice were bred, treated, and fed as described in Example 30. The accumulation of proteins (ubiquitin and protein tau) was determined as described in Example 27, except that protein levels were measured at 6 weeks and 8 months of age, i.e.,

Results:
Hippocampal quantification of ubiquitin aggregates as shown in Figure 12. The results are represented as the means ± the SEM. Bars with different letters are significantly different from each other (p < 0.05). The far left-hand white bar shows the level of ubiquitin aggregates in the brains of 8-month-old wild-type mice. The “Baseline” value shows the level at 6 weeks of age in the A53T mice. The black bar, second from right, shows the level of ubiquitin aggregates in the brains of 8-month-old untreated A53T mice (control). The right-hand grey bar shows the level of ubiquitin aggregates in the brains of 8-month-old A53T mice treated with Compound 1.

The images in Figure 13 show ubiquitin immunoreactivity in the hippocampi of 8-month-old A53T mice, either untreated control mice (Fig. 13A) or mice treated with Compound 1 (Fig. 13B).
Hippocampal quantification of protein tau aggregates is shown in Figure 14. The results are represented as the means ± the SEM. Bars with different letters are significantly different from each other (p < 0.05). The far left-hand white bar shows the level of protein tau aggregates in the brains of 8 month old wild-type mice. The “Baseline” value shows the protein level at 6 weeks of age in the A53T mice. The black bar, second from right, shows the level of protein tau aggregates in the brains of 8 month old untreated A53T mice (control). The right-hand grey bar shows the level of protein tau aggregates in the brains of 8 month old A53T mice treated with Compound 1.

Thus, treatment with quinuclidine compounds as described herein can reduce the accumulation of protein aggregates in the brains of A53T mice. In particular, a significant reduction in the level of protein tau aggregates is observed in mice treated with Compound 1.

Example 32: Administration of Compound 1 reverses memory aberrations of post-symptomatic \( \text{Gba1}^{D409V/D409V} \) mice. The ability of quinuclidine compounds as described herein to correct the biochemical and memory deficits of symptomatic \( \text{Gba1}^{D409V/D409V} \) mice was assessed. Ed.

Methods:

\( \text{Gba1}^{D409V/D409V} \) mice were bred and treated according to Example 24. Mice were fed the control diet or the Compound 1 diet as described in Example 24, except that drug ug
Hippocampal memory was evaluated with the NOR test according to Example 25, except that the mice were tested at 6 months of age (before treatment) and again 6 months later, i.e., at 12 months of age (after treatment).

**Results:**

Testing of the Gba1<sup>D409V/D409V</sup> mice before treatment confirmed that they exhibited impaired novel object recollection (not shown). The results of the NOR test at 12 months (after treatment) are shown in Figure 16. The results are represented as the means ± the SEM. The horizontal line demarcates 50% target investigations, which represents no preference for either object.

Age-matched wild-type mice (left-hand black bar) investigated the novel object significantly more frequently (n=13, p<0.01). In contrast, untreated Gba1<sup>D409V/D409V</sup> mice (middle white bar) showed no preference for the novel object, indicating a cognitive impairment. Symptomatic Gba1<sup>D409V/D409V</sup> mice treated with Compound 1 (right-hand grey bar) recovered the ability to investigate the unfamiliar object during the testing trial (n=13, p<0.05).

These results demonstrate that administration of quinuclidine compounds as described herein can reverse memory aberrations associated with proteinopathies, even when administration is initiated after symptoms of the proteinopathy are observed.

Example 33: Administration of Compound 1 reduces protein aggregation in the brains of post-symptomatic Gba1<sup>D409V/D409V</sup> mice. The ability of quinuclidine compounds as described herein to reduce and/or reverse these protein aggregations in the brains of symptomatic Gba1<sup>D409V/D409V</sup> mice is assessed.
**Methods:**

Wild-type (WT) and Gba1<sup>D409V/D409V</sup> mice are bred and treated substantially as described in Example 27, except that drug administration is initiated when animals are symptomatic for cognitive impairment, e.g., at approximately 6 months of age, i.e.,...

The accumulation of proteins (ubiquitin, D-synuclein and protein tau) is determined by hippocampal quantification and protein immunoreactivity substantially as described in Example 27.

**Results:**

Administration of quinuclidine compounds as described herein, e.g., Compound 1, is expected to lead to a measurable reduction in the accumulation of protein aggregates (ubiquitin, D-synuclein and/or protein tau) in the brains of Gba1<sup>D409V/D409V</sup> mice, even when drug administration is initiated after symptoms of cognitive impairment are observed.

Example 34: Administration of Compound 2 improves memory deficit in Gba1<sup>D409V/D409V</sup> mice.

The ability of quinuclidine compounds as described herein to improve memory deficit in Gba1<sup>D409V/D409V</sup> mice is evaluated using novel object recognition (NOR) and fear conditioning (FC) tests.

**Methods:**

Gba1<sup>D409V/D409V</sup> mice are bred and treated substantially as described in Example 24. Mice are fed a control diet or a diet containing a quinuclidine compound substantially as described in Example 25, except that the compound administered is Compound 2.

The NOR test and the FC test are performed substantially as described in Example 25.

**Results:**

Administration of Compound 2 is expected to improve memory deficit in Gba1<sup>D409V/D409V</sup> mice.
Example 35: Administration of Compound 2 reduces protein aggregation in the brain.

The ability of quinuclidine compounds as described herein to reduce and/or reverse protein aggregation in the brains of Gba1 D409V/D409V mice is assessed.

Methods:

Wild-type (WT) and Gba1 D409V/D409V mice are fed a control diet or a diet containing a quinuclidine compound substantially as described in Example 27, except that the compound administered is Compound 2.

The accumulation of proteins (ubiquitin, D-synuclein and protein tau) is determined by hippocampal quantification and protein immunoactivity as described in Example 27.

Results:

Administration of quinuclidine compounds as described herein, e.g. Compound 2, is expected to lead to a measurable reduction in the accumulation of protein aggregates (ubiquitin, D-synuclein and/or protein tau) in the brains of Gba1 D409V/D409V mice.

It is to be understood that while the invention has been described in conjunction with the above embodiments, that the foregoing description and examples are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

All publications, patent applications, patents, and other references mentioned herein are expressly incorporated by reference in their entirety, to the same extent as if each were incorporated by reference individually. In case of conflict, the present specification, including definitions, will control.
CLAIMS:

1. A method of treating a proteinopathy in a subject, the method comprising administering to the subject an effective amount of a compound of formula (I),

![Formula Image](image)

wherein:

- $R^1$ is hydrogen, a halogen, an acyano, nitro, hydroxy, thio or amino group, or a $C_{1-6}$-alkyl, $C_{2-6}$-alkenyl, $C_{2-6}$-alkynyl, $C_{1-6}$-alkyloxy, $C_{2-6}$-alkenyloxy or $C_{2-6}$-alkynyloxy group, optionally substituted by one or more (e.g., 1, 2, or 3) groups independently selected from a halogen, and a cyano, nitro, hydroxy, thio, or amino group;
- $R^2$ and $R^3$ are each independently selected from a $C_{1-3}$-alkyl group, optionally substituted by one or more halogens, or $R^2$ and $R^3$ together form a cyclopropyl or cyclobutyl group, optionally substituted by one or more halogens;
- $R^4$, $R^5$, and $R^6$ are each independently selected from hydrogen, a halogen, a nitro, a hydroxy, thio or amino group, and an $C_{1-6}$-alkyloxy or $C_{1-6}$-alkenyloxy or $C_{1-6}$-alkynyloxy group;
- $A$ is a 5- or 6-membered aryl or heteroaryl group.

2. The method of claim 1, wherein $R^1$ is hydrogen, a fluorine, an methyl or ethyl group, optionally substituted by a halogen, a hydroxy, thio or amino group.

3. The method of claim 1 or 2, wherein $R^2$ and $R^3$ are each independently selected from methyl and ethyl groups, optionally substituted, with one or more fluorine atoms.
4. The method of any one of claims 1 to 3, wherein R₁ is selected from a halogen; and a C₁₋₃-alkyl or C₁₋₃-alkyloxy group, optionally substituted by one or more groups selected from a halogen and a C₁₋₃-alkyloxy group.

5. The method of any one of claims 1 to 4, wherein R¹ and R² are both hydrogen.

6. The method of any one of claims 1 to 5, wherein R₁ is fluorine or a 2-methoxyethoxy group, and R² and R³ are hydrogen.

7. The method of any one of claims 1 to 6, wherein R₁ is in a position on the benzene ring para to the group A.

8. The method of any one of claims 1 to 7, wherein A is benzyl, optionally substituted with 1, 2 or 3 groups independently selected from a halogen, an hydroxy, a thio, an amino, a nitro, a oxo or a methyl group.

9. The method of claim 8, wherein the groups: C(R¹ R²)⁻¹ and -(C₂H₂R³ R⁴) are attached to group A in a 1,3- or a 1,4-relationship.

10. The method of any one of claims 1 to 7, wherein A is a 5-membered heteroaryl group which contains 1 or 2 heteroatoms selected from N and S, S.

11. The method of claim 10, wherein the groups: C(R¹ R²)⁻¹ and -(C₂H₂R³ R⁴) are attached to group A in a 1,3-relationship.

12. The method of any one of claims 1 to 11, wherein said compound is a compound of formula (II), (III) or (IV),

![Chemical Structure Image](image-url)
or a pharmaceutically acceptable salt or prodrug thereof.

13. The method of claim 12, wherein said compound is a compound of formula (V), (VI), (VII), or (VIII), or a pharmaceutically acceptable salt or prodrug thereof.

14. The method of any one of claims 1 to 11, wherein said compound is a compound of formula (VI), (VII) or (VIII), or a pharmaceutically acceptable salt or prodrug thereof.
or a pharmaceutically acceptable salt or prodrug thereof.

15. The method of claim 14, wherein said compound is a compound of formula (IX) or (XI), or a pharmaceutically acceptable salt or prodrug thereof.

15. The method of claim 14, wherein said compound is a compound of formula (IX) or (XI), or a pharmaceutically acceptable salt or prodrug thereof.
or a pharmaceutically acceptable salt or prodrug thereof.

16. The method of claim 15, wherein $R^4$ is fluorine.

17. The method of claim 1, wherein said compound is selected from: quinuclidin-3-yl (2-(4'-fluoro-[1,1'-biphenyl]-3-yl)propan-2-yl)carbamate; (S)-quinuclidin-3-yl (2-(2-(4-(4-fluorophenyl)thiazol-4-yl)propan-2-yl)carbamate; (S)-quinuclidin-3-yl (2-(4'-2-(2-(4'-methoxyethoxy)-[1,1'-biphenyl]-4-yl)propan-2-yl)carbamate; and the pharmaceutically acceptable salts and prodrugs thereof.

18. The method of any one of claims 1 to 17, wherein said proteinopathy is a tauopathy.

19. The method of claim 18, wherein said tauopathy is selected from: Parkinson's disease, Alzheimer's disease, Lewy Body Dementia, Pick's disease, progressive supranuclear palsy, dementia pugilistica, Parkinsonism linked to chromosome 17, Lyticoc-Bo-dig disease, tangle predominant dementia, Argyrophilic grain disease, ganglioglioma, gangliocytoma, meningoangiomatosis, subacute sclerosing panencephalitis, lead.
encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, lipofuscinosis, corticobasal degeneration, frontotemporal dementia, frontotemporal lobar degeneration and Huntington’s disease.

20. The method of claim 18 or 19, wherein said subject does not have protein aggregates comprising α-synuclein in their CNS (e.g. in neurons of the substantia nigra, cerebral cortex, hippocampus, frontal lobes and/or temporal lobes).

21. The method of claim 18, wherein said tauopathy is Parkinson’s disease characterised by the presence of protein tau, but not α-synuclein, within protein aggregates in the CNS of said subject (e.g. in neurons of the substantia nigra, cerebral cortex, hippocampus, frontal lobes and/or temporal lobes).

22. The method of any one of claims 1 to 17, wherein said proteinopathy is as a synucleinopathy.

23. The method of claim 22, wherein said synucleinopathy is selected from Lewy Body Dementia, Parkinson’s disease and multiple system atrophy.

24. The method of any one of claims 1 to 23, wherein said method prevents, reduces or reverses the progression of dementia in the subject.

25. The method of any one of claims 1 to 24, wherein said subject is a mammal, e.g. a human.

26. The method of any one of claims 1 to 25, wherein said subject has been diagnosed as being at risk of developing said proteinopathy, and wherein the method prevents, or delays, the onset and/or development of the proteinopathy in the subject.

27. The method of any one of claims 1 to 26, wherein said compound, or pharmaceutically acceptable salt or prodrug thereof, is administered by systemic or oral administration, e.g. via a non-parenteral route.

28. The method of claim 27, wherein said compound, or pharmaceutically acceptable salt or prodrug thereof, is administered orally.
29. A compound, or a pharmaceutically acceptable salt thereof, as defined in any one of claims 1 to 17 for use in a method of treating a proteinopathy in a subject.

30. The compound for use according to claim 29, wherein said method of treating a proteinopathy is as defined in any one of claims 18 to 28.

31. Use of a compound, or a pharmaceutically acceptable salt thereof, as defined in any one of claims 1 to 17 in the manufacture of a medicament for use in an a method of treating a proteinopathy in a subject.

32. The use of claim 31, wherein said method of treating a proteinopathy is as defined in any one of claims 18 to 28.

33. A method of reducing, reversing or preventing the accumulation of protein in aggregates in tissue of a subject diagnosed as having a proteinopathy, or diagnosed as being at risk of developing a proteinopathy, wherein said protein aggregates comprise α-synuclein, the method comprising administering to said subject an effective amount of a compound, or a pharmaceutically acceptable salt, or prodrug thereof, as defined in any one of claims 1 to 17.

34. The method of claim 33, wherein said protein aggregates are aggregates of protein in tau, and wherein said proteinopathy is a tauopathy.

35. The method of claim 34, wherein said tauopathy is selected from Parkinson’s disease, Alzheimer’s disease, Lewy Body Dementia, Pick’s disease, progressive supranuclear palsy, dementia pugilistica, parkinsonism linked to chromosome 17, Lysitico-Bodig disease, tangle predominant dementia, argyrophilic grain disease, ganglioglioma, gangliocytoma, meningoangiomatosis, subacute sclerosing panencephalitis, lead, ad encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, lipofuscinosis, AD, corticobasal degeneration, frontotemporal dementia, frontotemporal lobar degeneration and Huntington’s disease.

36. The method of any one of claims 33 to 35, wherein said subject does not have α-synuclein in said tissue.

37. The method of claim 35 or 36, wherein said tauopathy is Parkinson’s disease.
38. The method of claim 33, wherein said protein aggregates are aggregates of a-synuclein and wherein said proteopathy is a synucleinopathy.

39. The method of claim 38, wherein said synucleinopathy is selected from Lewy Body Dementia, Parkinson's disease and multiple system atrophy.

40. The method of any one of claims 33 to 39, wherein said method prevents, reduces or reverses the progression of dementia in the subject.

41. The method of any one of claims 33 to 40, wherein said tissue is a neuron of the substantia nigra, cerebral cortex, hippocampus, frontal lobes and/or temporal lobes of said subject.

42. The method of any one of claims 33 to 41, wherein said subject is a mammal, e.g. a human.

43. The method of any one of claims 33 to 42, wherein said compound, or pharmaceutically acceptable salt or prodrug thereof, is administered by systemic administration, e.g. via an non-parenteral route.

44. The method of claim 43, wherein said compound, or pharmaceutically acceptable salt or prodrug thereof, is administered orally.

45. A method of preventing, reducing or reversing loss of neural function in a subject diagnosed as having, or at risk of developing, a proteinopathy, the method comprising administering to said subject an effective amount of a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined in any one of claims 1 to 17.

46. The method of claim 45, wherein said proteopathy is a tauopathy.

47. The method of claim 46, wherein said tauopathy is selected from Parkinson's disease, Alzheimer's disease, Lewy Body Dementia, Pick's disease, progressive supranuclear palsy, dementia pugilistica, parkinsonism linked to chromosome 17, Lytic-Bodig disease, tangle predominant dementia, Argyrophilic grain disease, ganglioglioma, gangliocytoma, meningioangiomatosis, subacute sclerosing panencephalitis, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease, lipofuscinosis,
corticobasal degeneration, frontotemporal dementia, frontotemporal lobar degeneration and Huntington's disease.

48. The method of any one of claims 45 to 47, wherein said subject does not have protein aggregates comprising α-synuclein in their CNS (e.g., in neurons of the substantia nigra, cerebral cortex, hippocampus, frontal lobes and/or temporal lobes).

49. The method of claim 47, wherein said tauopathy is Parkinson's disease characterised by the presence of protein tau, but not α-synuclein, within protein aggregates in the CNS of said subject (e.g., in neurons of the substantia nigra, cerebral cortex, hippocampus, frontal lobes and/or temporal lobes).

50. The method of claim 45, wherein said proteinopathy is synucleinopathy.

51. The method of claim 50, wherein said synucleinopathy is selected from Lewy Body Dementia, Parkinson's disease and multiple system atrophy.

52. The method of any one of claims 45 to 51, wherein said method prevents, reduces or reverses the progression of dementia in the subject.

53. The method of any one of claims 45 to 52, wherein said subject is a mammal, e.g., a human.

54. The method of any one of claims 45 to 53, wherein said compound, or pharmaceutically acceptable salt or prodrug thereof, is administered by systemic administration, e.g., via a non-parenteral route.

55. The method of claim 54, wherein said compound, or pharmaceutically acceptable salt or prodrug thereof, is administered orally.

56. The method of any one of claims 45 to 55, wherein the loss of neural function comprises loss of cognitive function, autonomic function and/or motor function.

57. The method of claim 56, wherein the loss of neural function comprises loss of cognitive function.
58. The method of claim 57, wherein the method prevents, reduces or reverses deterioration in cognitive domains in the subject.

59. The method of claim 58, wherein the method prevents, reduces or reverses deterioration in attention and concentration, executive functions, memory (e.g. working memory), language, visuo-constructional skills, conceptual thinking, calculations, ns, orientation, decision making and/or problem solving.

60. The method of any one of claims 56 to 59, wherein the loss of neural function comprises loss of autonomic function and the method prevents, reduces or reverses orthostatic hypotension, constipation, dysphagia, nausea, hypersalivation, hyperhidrosis and/or urinary and sexual dysfunction.

61. The method of any one of claims 56 to 60, wherein the loss of neural function comprises loss of motor function and the method prevents, reduces or reverses Parkinsonism.

62. The method of claim 61, wherein the method prevents, reduces or reverses motor dysfunction (e.g. tremor), bradykinesia, rigidity, postural instability and/or impaired balance.

63. A compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined in any one of claims 1 to 17, for use in a method of preventing, reducing or reversing loss of neural function in a subject as claimed in any one of claims 45 to 62.

64. Use of a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined in any one of claims 1 to 17 in the manufacture of a medicament for use in an a method of preventing, reducing or reversing loss of neural function in a subject as claimed in any one of claims 45 to 62.

65. A method of preventing, reducing or reversing the progression of dementia in a subject diagnosed as having, or at risk of developing, a proteinopathy, the method comprising administering to the subject an effective amount of compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined in any one of claims 1 to 17.
66. The method of claim 65, wherein the method prevents, reduces or reverses early rly symptoms of dementia (e.g. difficulty remembering recent conversations, names or of events, and/or apathy and depression).

67. The method of claim 65 or 66, wherein the method prevents, reduces or reverses ses later symptoms of dementia (e.g. impaired communication, poor judgment, disorientation, on, confusion, behavior changes and/or difficulty in speaking, swallowing and/or walking). g.

68. A compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined in any one of claims 1 to 17 for use in a method of preventing, reducing or reversing the progression of dementia in a subject as claimed in any one of claims 65 to 67.7.

69. Use of a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined in any one of claims 1 to 17 in the manufacture of a medicament for use in an a method of preventing, reducing or reversing the progression of dementia in a subject as claimed in any one of claims 65 to 67.7.

70. A method of preventing, reducing or reversing mild cognitive impairment in an a subject diagnosed as having, or at risk of developing, a proteinopathy, the method of comprising: administering to the subject an effective amount of compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined in any one of claims 1 to 17.

71. A compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined in any one of claims 1 to 17 for use in a method of preventing, reducing or reversing mild cognitive impairment in a subject as claimed in claim 70.

72. Use of a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined in any one of claims 1 to 17 in the manufacture of a medicament for use in an a method of preventing, reducing or reversing mild cognitive impairment in a subject as claimed in claim 70.

73. A pharmaceutical dosage form comprising a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined in any one of claims 1 to 17, and a pharmaceutically acceptable excipient.
wherein the dosage form is formulated to provide, when administered orally, an amount of said compound, salt or prodrug sufficient to prevent, reduce or reverse the accumulation of protein aggregates in tissue of a human subject diagnosed as having, or at risk of developing, a proteinopathy.

74. The pharmaceutical dosage form of claim 73, wherein said dosage form is formulated to provide, when administered orally, an amount of said compound, salt or prodrug sufficient to prevent, reduce or reverse the accumulation of protein tau-containing aggregates in tissue of a human subject diagnosed as having, or at risk of developing, Parkinson’s disease.

75. The pharmaceutical dosage form of claim 73, wherein said dosage form is formulated to provide, when administered orally, an amount of said compound, salt or prodrug sufficient to prevent, reduce or reverse the accumulation of \( \alpha \)-synuclein-containing aggregates in tissue of a human subject diagnosed as having, or at risk of developing, Lewy Body Dementia.

76. The pharmaceutical dosage form of any one of claims 73 to 75, wherein said dosage form is a neuron of the substantia nigra, cerebral cortex, hippocampus, frontal lobes, and/or temporal lobes.

77. The pharmaceutical dosage form of any one of claims 73 to 76, wherein said dosage form comprises a further agent which is capable of treating or preventing said proteinopathy.

78. A pharmaceutical composition comprising: (i) a compound, or a pharmaceutically acceptable salt or prodrug thereof, as defined in any one of claims 1 to 17; (ii) a further agent which is capable of treating or preventing a proteinopathy; and (iii) a pharmaceutically acceptable excipient.

79. The pharmaceutical composition of claim 78, wherein said further agent is selected from: a dopamine precursor (e.g., l-DOPA), a dopamine agonist (e.g., bromocriptine, cabergoline, pergolide, pramipexole, or apomorphine), an MAO-B inhibitor (e.g., rasagiline, selegiline), an anticholinergic (e.g., orphenadrine, procyclidine), or...
trihexyphenidyl), an enhancer of \( \alpha \)-glucocerebrosidase activity (e.g., ambroxol or afegostat) and lamantadine.

80. The pharmaceutical composition of claim 78, wherein said further agent is an acetylcholinesterase inhibitor (e.g., tacrine, rivastigmine, galantamine, donepezil, or memantine).

81. The pharmaceutical composition of any one of claims 78 to 80, wherein said further agent is a tauopathy selected from Parkinson's disease, Alzheimer's disease, Lewy Body Dementia, Pick's disease, progressive supranuclear palsy, dementia pugilistica, parkinsonism linked to chromosome 17, Lytico-Bodig disease, argyrophilic grain disease, ganglioglioma, gangliocytoma, anaemia, meningoangiomatosis, subacute sclerosing panencephalitis, laddencephalopathy, ype, tuberous sclerosis, Hallervorden-Spatz disease, dipofusinosis, corticobasal degeneration, on, frontotemporal dementia, frontotemporal lobar degeneration, and Huntington’s disease.

82. The pharmaceutical composition of claim 81, wherein said tauopathy is Parkinson's disease.

83. The pharmaceutical composition of any one of claims 78 to 80, wherein said further agent is a synucleinopathy selected from Lewy Body Dementia, Parkinson's disease and multiple system atrophy.

84. The pharmaceutical composition of any one of claims 78 to 83, wherein said further agent is formulated for systemic administration, e.g., via a non-parenteral route.

85. The pharmaceutical composition of claim 84, wherein said composition is formulated for oral administration.

86. The pharmaceutical dosage form of any one of claims 73 to 77, or the pharmaceutical composition of any one of claims 78 to 85, for use in therapy.

87. The pharmaceutical dosage form of any one of claims 73 to 77, or the pharmaceutical composition of any one of claims 78 to 85, for use in a method as defined in any one of claims 71 to 72.
FIG. 3
FIG. 4
FIG. 7
FIG. 8
FIG. 9
FIG. 11
FIG. 13
FIG. 16
**INTERNATIONAL SEARCH REPORT**

International application No
PCT/US2016/021512

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/00 A61K31/439
ADD. A61P25/16 A61P25/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>claims 253, 254; claims 266, 293 ----</td>
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* Further documents are listed in the continuation of Box C. X See patent family annex.

* Special categories of cited documents:

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"E" earlier application or patent but published on or after the International filing date

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search
13 May 2016

Date of mailing of the international search report
23/05/2016

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer
Dahse, Thomas

Form PCT/ISA210 (second sheet) (April 2009)
**INTERNATIONAL SEARCH REPORT**

International application No

PCT/US2016/021512

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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## INTERNATIONAL SEARCH REPORT

**Information on patent family members**

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